BIOREMEDIATION AND SEQUESTRATION OF MICROPLASTICS IN THE AQUEOUS ENVIRONMENT

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Abstract

Accumulation of microplastics has continued to plague our water sources, land, and air. The continued accumulation of these plastics can cause detriments to the human body and other living organisms. The findings of this experiment can conclusively deduce that the addition of the fungus cultures *A.Niger* and *P.Microspora* do, in fact, have a statistically significant effect on the reduction and bioremediation in the mass of polyurethane particulates. This can be observed by the p-values of .032 and .031 respectively which resulted in a rejection of the null hypothesis at the α=.05 significance level. In addition, the mechanical multi-level filtration system was observed to have 89% effectiveness at sequestration of polyurethane particulate in 3 trials. Specifically, this experiment analyzes the effect of the addition of *P.Microspora* and *A.Niger* to 1.0g samples of polyurethane particulate for significant mass reduction. Furthermore, this experiment will investigate the application of these methods to reduce plastics wastes on a small scale through bioremediation, as well as methods to sequester them.

Literature Review

The long-standing issue of our plastics in the ocean is a rising concern across the world. With global utilization of plastics at an all-time high, what are we to do with the wastes that are produced? Although we use plastics in vast quantities we have no solution to the proper disposal of these materials. To situate this issue, we must understand what microplastics are and how they form before we can observe the methodology of breaking them down. “Microplastics (particles with a diameter <1 mm, with no lower limit) derive from progressive fragmentation of larger plastic items or may be manufactured to be a small size for use in personal care products, medicines, and industry.” (Galloway & Lewis 233) Continuous fragmentation of microplastics within all environments and will eventually reach a consumer level that can reach humans and enter their bodies. As these microplastics break down there are various effects on the environment and on humans. As asserted by Galloway and Lewis,

> “The impacts of plastic ingestion in laboratory studies include gut blockage and physical injury, oxidative stress, altered feeding behavior, and reduced energy allocation, with knock-on effects for growth and reproduction. Transfer to tissues of plastics associated with chemicals, many of which possess endocrine-disrupting activity, adds to the potential toxicity of ingested particles through activation of signal transduction pathways relevant to hormone action.” (Pg 2231)

The particulate nature of these plastics has the potential and ability to affect the lives of humans negatively impacting their physical health and even possibly affecting their mental health, as it disrupts the endocrine system which controls the production of hormones vital to the proper functioning of our bodies and affects mood. Furthermore, microplastics have been

detected concurrently proving the fact that microplastics are already passing through human digestive systems, “Evidence of microplastics being detected in humans for the first time. Every single sample examined in the study contained microplastic; in some cases, nine different types of plastic were found in just one sample. On average, the researchers discovered 20 microplastic particles per 10g of human waste” (Shute). This can have detrimental effects on human health which are evident, as our consumptions of these harmful plastics increase as our lives continue onward. Moreover, microplastics can be detected in the airborne atmosphere, “We kind of expected to find plastics there, but we certainly were not prepared for the numbers we found. It was astounding: 11,400 pieces of microplastic per square meter per month, on average” (Stack 1). Having situated the number of microplastics per square meter we can understand that the amount of exposure doesn't only come from ingestion but also our breathing, furthering the point that there isn’t much we can do to prevent the consumption. Accordingly, there are more health consequences associated, “The chemicals that make up the plastic, we know they have an impact on the animal endocrine system and the lymphatic system, which regulate the production of hormones and the elimination of bodily toxins” (Stack). Without the removal of bodily toxins, the homeostasis of humans can be thrown into chaos and could potentially lead to death. Furthering the idea of toxins entering the body,

> “Experts fear the presence of microplastics in the body may damage the immune system, trigger inflammation and help carry toxins such as mercury or pesticides. Sadly, it is not as simple as making lifestyle changes like cutting fish from our diets, ditching cotton or switching from tap to bottled water, as microplastics have permeated every aspect of modern life”(Shute).

Specific toxins such as mercury and pesticides are commonly known, to affect the immune system, lungs and even cause kidney failure. Microplastics effectively suppress our

immune response to dealing with these harmful substances and can accumulate in the body leading to toxicosis or even death. The neverending list of complications seems incessant, yet consequently, our actions affect other biodiversity, as humans place this strain and many ecosystems that thrive in clean environments.

Aquatic and Marine life is constantly affected by microplastics and this injurious issue. Entering the food chain microplastics introduce an unnatural substrate that biodiversity is unequipped to deal with or break down. Nsikan Akpan, a science editor at National Geographic, affirms “The longer that these plastics reside in these organs, the higher the chance that plastics will end up in the food chain from crabs on to other animals.” (9) As plastics work their way up the food chain they will eventually reach humans as primary consumers of crabs include humans, octopuses and otters. (Akpan 9) Humans, being the top of the food chain, leaves them susceptible to the high levels of absorption of these plastics, further leading to unnecessary consumption of microplastics. A rising concern of populations on the decline in marine environments have been increasingly concerning as microplastics can decrease the fertility of shellfish such as Oysters. “After 2 months, exposed oysters had significant decreases in oocyte number (−38%), diameter (−5%), and sperm velocity (−23%). The D-larval yield and larval development of offspring derived from exposed parents decreased by 41% and 18%, respectively, compared with control offspring.”(Sussarellu) The breeding of Oysters has a negative correlation on its breeding patterns; when microplastics are introduced to the existing water supply leading to lower fertility rates. Decreases in fertility rates of these Oysters can serve a multitude of issues as it could lead to the elimination of Oyster and shellfish populations as infertility becomes generational. Accordingly, this decrease in the number of Oysters can lead to less sustainability and increase

instability within an ecosystem as they face a depleting primary food source. The detrimental effects that microplastics will have on marine biodiversity, can be observed as strictly worsening with no plausible solution to remove the entry of microplastic particles into waterways. Although much of our marine life is at risk there is certain marine life such as the Danafungia Scrupulosa, a marine plate coral that is able to sequester these plastics but has not proven efficacy of bioremediation. (Jambeck 3) Such plate corals serve as a field for future exploration and mimicry simulations utilizing filtration systems similar to that of corals are plausible solutions to removing plastic pollution in their environments. Through active or passive sequestration these corals act like vacuums pumping water in and can be similarly recreated with a mechanical pump and filter. Thus, the need for viable options for bioremediation and sequestration mechanisms of these plastics are necessary to restore balance to these ecosystems.

Plastics on coastal sediments have been a common occurrence in all parts of the world however are not easily visible to the naked human eye yet exist in substantial quantities. These plastics have various harmful effects such as stated by Kara Lavender Law an Oceanographer and Richard C. Thompson a Marine Biologist, " In coastal sediments around the world microplastics also appear to be ubiquitous with quantities typically ranging from 3 to 30 particles per 250 ml of sediment." (145) The quantities of such plastics serve as a basis to situate the general makeup of coastal sediments, thus illustrating the effect that these plastics have had on coastal sediments. Moreover, with such high quantities of plastics, in 70 years there will be such a problem that it will no longer just be in some parts of the world but a global ocean crisis. (Microplastics...food chain 1) With this rise in global plastic accumulation, the consequences of our actions can be only be described as insurmountable, "In the ocean, they either settle out

slowly or can be transported rapidly by episodic turbidity currents - powerful underwater avalanches - that travel down submarine canyons to the deep seafloor. Once in the deep sea, microplastics are readily picked up and carried by continuously flowing seafloor currents that can preferentially concentrate fibers and fragments within large drifts of sediment." (Scientists...seafloor) The issue has reached such intractability that we have begun to see the sediments accumulate at the seafloor. Specific instances, such as observed in the Great Pacific Garbage Patches, have created ocean gyres that alter the composition of the water and ocean floor elucidated as a sludge-like substance creating a murkiness and unsafe water condition. (Scientists... seafloor 2) The understanding of the microplastics that have accumulated around the world is virtually immeasurable due to the shear effect that has begun to have in global waters. Thus leading us to the solution of riding the ocean of microplastics.

We have observed the effects that microplastics have put on the world from causing harm to humans and to that of animals, in aquatic and land-hailing habitats. The issue at hand will only worsen. Nextly, the accumulations are becoming overwhelming to an extent to which we will begin to see the issue becoming globalized if a solution verdict has not been reached. Although, the stakes of such a widespread issue have arisen not only locally in waters, but begun to become global. There is a solution, the bioremediation of microplastics through fungi. Microorganisms such as fungi have been proven an efficacious approach to solve our plastic crisis, not only on land but can surely be applied to the aqueous environment. Sequestering the plastics utilizing corals, and remediating, later on, seem to be the greatest solution to collection and disposal that we have developed to our blind eye in the accumulation of this waste. Specifically, species such as *Pestalotiopsis Microspora* are proven to have broken down polyurethanes, an extremely

common type of microplastic accumulating in oceans across the world. (Russell 1) With bioremediation using specific fungi we can bring an end to the plastics that plague us with their seemingly endless supply as we produce more and more plastic goods, each and every day.

The investigation of these topics led to the research of the bioremediation and sequestration of the microplastics waste polyurethane. Conducted using the fungal species *P.microspora* and *A.niger*, in addition, the sequestration of these plastics will use a multilevel filtration system

Methodology

The methodology conducted in this experiment will mainly be content analysis, in which this experiment will be easily replicated and inferences can be made through analysis of numerical quantitative data. Furthermore, this experiment involves quantitative research and with a directive in understanding the efficiency of sequestration and bioremediation of polyurethane microplastic particulates. Moreover, the utilization of the multi level filtration system, consisting of two filter papers and a 12x12 cheesecloth encased funnel will allow us to measure within a 2-gallon capacity, the effectiveness of the coral simulation to effectively and efficiently sequester approximately +/- 15 percent of all added mass. Each trial will be run with a limitation of 2L of water filtered through the pump and recorded to observe effective mass sequestration on the filter. Utilizing a baseline measurement of a pure 2L sample will allow us to observe any existent particulate in the water supply. After collecting the mass of the filter before and after 3 trials we can then average the differences in sample and record the reduction in the polyurethane within the tank. Furthermore, the methodology will include stained pieces of the

particulate to make tracking the movement of particles more apparent. All conditions of the tanks will be kept constant between both tanks with an average of 0ppm ammonia, the specific gravity of (1.024-1.026), Nitrite 0ppm, Nitrate <10ppm, Calcium 420-440ppm, Alkalinity of (8-9.5 dKH), and Magnesium 1260-1350ppm. The conditions met for the water are to ensure ideal conditions of corals in their natural environment and ensure any change in mass of polyurethane particulate sequestered is due to strictly the multilevel filtration system. These factors will be measured each day and kept constant to ensure there are no variances in the samples of water that would alter the conditions in which the polyurethane exists. In addition, a control group will be run in order to account for any existent particulate in the water supply. All polyurethane particles will be less than <5mm as this is the size consideration for the size of microplastics. After each trial, the corals will be transferred to a tank with the same water conditions as before but removed from exposure to the polyurethane to reject the possibility of extended exposure. All trials run by volume rather than a time period and results will be entered into a data table and mean calculations will be used to average the amount sequestered after each week, in addition to the mean calculations standard deviations will be calculated to account for any errors in the data to create an interval of error.

The second half of this experiment will also include content analysis that provides data collected on the bioremediation of polyurethane particles <5 mm in size. Utilizing the fungus cultures *A.niger and P.microspora* we will measure the change in mass in the particulates and a baseline mass of 1g of polyurethane will be added to each petri dish. Both cultures will be grown on potato dextrose agar to ensure there is no advantage in nutrient source allowing for greater growth and bioremediation of the polyurethane. The

masses of each petri dish will be weighed after each day to measure the reduction in mass. The temperatures will be kept constant within the incubator to ensure that the evaporation rate from the agar dishes remains constant as well as the fungus cultures can grow within favorable conditions. The fungus cultures will also be continually monitored for mass checks each day and averaged to see whether there is growth within the colonies due to the agar nutrient or if there is a significant effect on the growth due to the bioremediation of the polyurethane particles. For comparison of the data of average mass change, strictly potato dextrose agar plates with cultures *A.niger* and *P.microspora* trials will be conducted and weighed each day for the same period of time. These trials will serve as a baseline for mass change due to nutrient consumption and evaporation. The same methodology will be used to record data and input it into a data table, then using mean calculations there will be an average of the masses of the Petri dishes, as well standard deviations of the mean weight will be calculated to serve as margin of error in mass measurements. Lastly, calculations will be utilized to analyze 2 sample t-tests for means to yield statistical significance of the addition of the fungus cultures to the polyurethane particulate.

Hypothesis

To accurately measure the effectiveness of the results recorded, hypotheses must be created to establish the effectiveness of the addition of the variables. Respectively, the null hypothesis for this experiment is that there is not a statistically significant effect in the reduction of mass with the addition of a fungus culture. Whereas, the alternative hypothesis would be that there was a statistically significant effect in reduction of mass with the addition of a fungus

culture. This idea would be compared at a significance level of α=.05. This significance level would be reasonable to compare any p-value to prove the significance of the addition of this value displaying any true reduction in mass. The hypothesis for the sequestration of the polyurethane is we can expect that the pump will sequester +/- 15 percent of all polyurethane particulate that enters the system within a one gram sample or .15 grams. This was reasonable as a margin of error could occur due to factors such as faulty filters or inaccurate weighing devices.

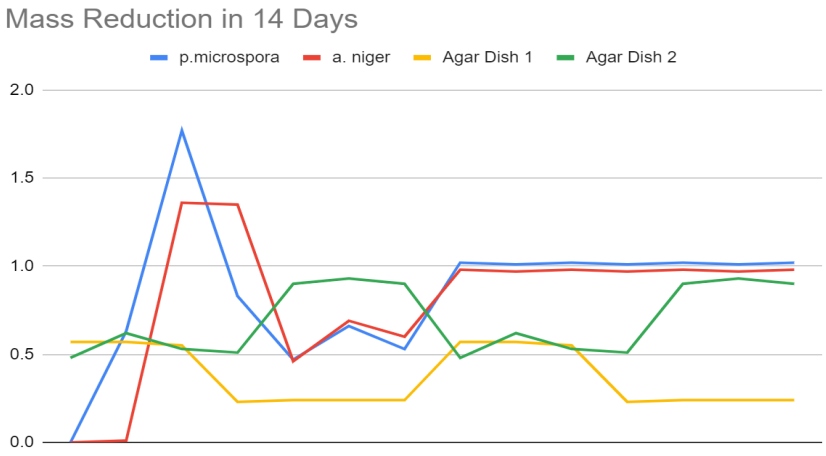
Results/Analysis

Collection of data revealed that on average there was a mean reduction of mass of about .857 grams per day over the course of a trial period of 14 days for *P.microspora*. In addition, there was an average decrease in mass of .807 grams for *A.niger*. To prove the efficacy of this methodology of bioremediation further data analysis was performed. Within the time constraint of the 14 day trial period, we yielded 14 mean data points per day.

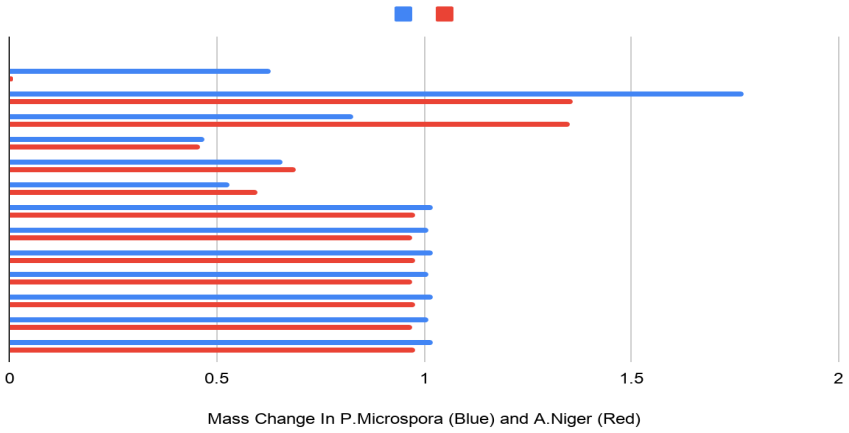
	p.microspora	a. niger	Agar Dish 1	Agar Dish 2
	0	0	0.57	0.48
	0.63	0.01	0.57	0.62
	1.77	1.36	0.55	0.53
	0.83	1.35	0.23	0.51
	0.47	0.46	0.24	0.9
	0.66	0.69	0.24	0.93
	0.53	0.6	0.24	0.9
	1.02	0.98	0.57	0.48
	1.01	0.97	0.57	0.62
	1.02	0.98	0.55	0.53
	1.01	0.97	0.23	0.51
	1.02	0.98	0.24	0.9
	1.01	0.97	0.24	0.93
	1.02	0.98	0.24	0.9
ST DEV	0.4011795794	0.4172700393	0.1674895437	0.1971221521
T-TEST means 1	0.031	0.032		
T-TEST means 2	0.031	0.032		

(Figure 1) Data points of 14 day trial for bioremediation with SD calculations and t-test p-values

Utilizing the data collected from the bioremediation trials (Figure 1), the standard deviation calculation was necessary in order to run further statistical analysis yielding a .401 data point for *P. microspora*. A .417 SD for *A.niger*, .167 Agar Control Dish 1, and .197 Agar Control Dish 2. Taking the means of the average reduction due to evaporation purely from control dishes was used to ensure statistical significance of true mean bioremediated mass. Then after calculating a 2 sample t-test for means in reference to control 1 and 2 with respect to each culture revealed, p values of .031 for *P.microspora* and .032 for *A.niger*. When compared to both controls they yielded statistically significant results at the .05 significance level. This led us to conclude that we reject the null and the alternative was true. There was a statistically significant mean reduction of polyurethane mass with the addition of the fungus cultures in both species *A.niger* and *P.microspora*. As depicted in the figure below there was a more significant reduction in mass between the plates with the addition of polyurethane as it served a competitive advantage as a food source and medium for growth over the span of the 14 days.



(Figure 2) Comparative Analysis of Fungus Cultures vs. Control Agar Dishes



(Figure 3) Comparison of mass change in *P.microspora* vs. *A.niger*

When comparing the two specific species of fungus against each other they had approximately the same mean reduction in mass over time ranging from 1-1.5% bioremediated on average after a complete daily cycle. The data demonstrates the understanding of constant mass reduction rather than spontaneous reduction removing error for improper weighing or inaccuracy in methodology. Moreover, it substantiates the argument that the polyurethane particulate is used as a medium and substrate for growth rather than remaining on top of the petri dish.

In the sequestration of the polyurethane particulate of three trials of 2L water, it was found that there was a mean total collected of .89 grams. This meant that the filtration system had approximately 89% effectiveness in sequestration of the particulate matter in the water supply. This led to the conclusion that there was a significant effect of adding this active filtration system to the tank in order to try to sequester these particulates. In addition to this finding, further ideas are presented that this pump can have application in still water aqueous environments such as lakes, ponds, and pools in order to reduce plastics that are existent in the current water supply. In addition to these limitations to this yielded result must be taken with

caution as there were not enough trials to effectively conclude that these results can be produced in a larger number of trials.

Discussion

After conducting this experiment obstacles held this experiment back from its full potential. Although it was run through a trial and error process it still resulted in favorable outcomes. Firstly, the original methodology included passive filtration utilizing the coral species. Fungia Repanda is a coral similar to that of Danafungia Scruposa, a previously researched coral used to passively sequester particulate matter in water. Finding a similar plate coral with many of the same characteristics would allow for the passive and biological filtration of the aqueous environment effectively solving two issues. Microplastics within its environment while also serving as a possible food source to further its growth and ability to thrive within its habitat. However, this proved difficult as it was extremely difficult to keep an organism that required specific care, maintenance, and specific conditions not only to thrive but simply survive. The conditions had to be perfect to the exact milliliter or factors such as alkalinity and nitrate concentration could cause bleaching. The issue at hand led to the eventual death of the corals, which created a new field of interest active sequestration through a multi level filtration system. As many prototypes were built to find an accurate solution, firstly gathering materials and arranging them a certain way would be necessary to effectively sequester these extremely fine microplastic particles and could easily pass through a material if it were too porous. Such materials in early prototypes included sponges, charcoal filters, and hot glue. This combination of these materials and the incorrect method to collect these plastics led to a less is more approach, in which more commonly found items were used such as cheesecloth and coffee

filters. This final model used a vacuum pump attached to a filter and clear tubing, the combination of these specific materials provided the perfect solution to sequester microplastics and small plastics wastes perfectly in any still water aqueous solution. Since these materials were extremely inexpensive many people would have adequate access to the materials. Therefore, increasing the efficacy of this method to reduce plastics existent in water supplies in areas that cannot access utilities such as electricity or afford a more expensive alternative. The filtration system created could be used by anyone ranging from children to elderly people which may be a limitation to more complicated and complex filtration systems.

Bioremediation of microplastics proved less difficult a task to head, as fungus cultures are natural decomposers and possess the ability to break down natural matter. Although some plastics are made of inorganic polymers, polyurethane is made of organic polymers making it a probable method for alleviating this stress humans are imposing on the environment. (Britannica 1) Certain decisions in the beginning trials exhibited issues in the initial approach to the issue. The initial approach to bioremediation was to culture *P.microspora* and *A.niger* directly on the polyurethane fragments however did not show any growth nor bioremediation of the particulate. Leading to a more traditional approach to culturing the polyurethane on potato dextrose agar plates. Once cultured on these plates the colonies began to grow and expand their grasp on the plates. Nextly, adding the polyurethane on top of the colonies would hopefully stimulate their need to colonize further. The extremely competitive nature of these fungi leads to the complete takeover of the polyurethane added to the plate. The fungi then began to use the polyurethane as a medium for further growth and proved to be a definitive approach to the elimination of polyurethane waste.

Conclusion

The bioremediation and sequestration of polyurethane particulate through the usage of *A.niger* and *P.microspora*, and the usage of a multilevel filtration system proved efficacy after extensive data collection. The expansive issues that come with plastic consumption and contamination in the marine environment can be solved using the methodologies used in this experiment. Primarily, the culturing of *A.niger* and *P.microspora* statistically significantly will reduce any added amounts of polyurethane on cultures potato dextrose agar plates. This solution can be utilized to break down small plastics wastes in small quantities such as plastic bottles, straws, and wrappers that are extremely common in consumer goods. Additionally, coastal sediments can be sequestered using this multilevel filtration system effectively collecting polyurethane particulate matter as it is an inexpensive and easily accessible solution to collecting plastic waste. Furthermore, in stillwater aqueous environments such as ponds, lakes, and pools this same method is applicable as it requires no electricity nor extra materials. The importance of utilizing these new approaches to the removal and reduction of plastic waste can lead us to a cleaner, greener, and brighter future for our planet and biodiversity.

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