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Effectiveness of *Pleurotus ostreatus* Mycelium at Filtering Nitrogen and Phosphorus from Solutions

KEY WORDS

Pleurotus ostreatus

mycofiltration

eutrophication

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ABSTRACT

Nutrient-dense runoff and eutrophication are causing widespread harm to the Chesapeake Bay's ecosystem. A solution is needed, or the problem will continue to worsen, putting more species in danger. Mycofiltration has been highlighted as an effective method for filtering heavy metals in contaminated drinking water. This study sought to design a mycofiltration system and determine if mycofiltration could also act as an effective technique for filtering nitrogen and phosphorus from contaminated runoff. If effective, mycelium could provide an affordable, scalable, and compostable solution to help manage nutrient pollution and eutrophication. This study involved designing and testing an experimental setup to filter solutions through mycelium and measure nutrient concentrations before and after filtering. This was accomplished by pouring nutrient-dense solutions through tubes of *Pleurotus ostreatus* mycelia and using spectrophotometry to measure absorbance. The results from this trial were ultimately inconclusive and justify running the experiment again with a few modifications. This would help determine conclusive results and add valuable information about whether or not mycofiltration is a technology worth exploring for phosphorus and nitrogen pollution control.

Introduction

The Chesapeake Bay is the largest watershed in the United States, spanning over 64,000 mi2, six states and the District of Columbia, and harboring over 3,600 unique species of plants and animals (Ollivier et al., 2024). Unfortunately, the Chesapeake has also felt some of the largest impacts from urbanization and agriculture. As a result, its health has drastically degraded over the past couple of decades. One key factor affecting the health of the bay is the total nitrogen and phosphorus content (Kleinman et al., 2019). While beneficial in the right quantities, too much of these nutrients can lead to problems. The main problem is eutrophication, in which nutrient-dense runoff encourages the growth of algae, leading to harmful algal blooms. These blooms block essential aquatic plants from photosynthesizing and can disrupt entire ecosystems. Furthermore, the algae can decrease the amount of dissolved oxygen in the water, a phenomenon known as hypoxia, which makes it difficult for other species to survive (Beegle, 2014; Testa et al., 2017; Boesch et al., 2001). Anthropogenic eutrophication is a global crisis, but the Chesapeake Bay is the most impacted watershed on the planet (Malone et al., 2020). As more land is developed, total nitrogen and phosphorus levels are projected to grow (Roberts et al., 2010). This will further the problem of eutrophication and threaten the species that rely on the Chesapeake Bay's resources.

It is clear that to reverse the negative trajectory of the bay, an affordable and sustainable solution is needed to help lower nutrient levels in runoff. In the past few decades, fungi have been praised for their decompositional properties, such as the ability to break down hydrocarbons and absorb heavy metals, and they have been put forward as a potential tool to aid in bioremediation and pollution reduction (Akpaja et al., 2014; Mehta et al., 2017). Prior studies have shown great potential in using mycelium as a filter for heavy metal-contaminated drinking water (Akpaja et al., 2014). Mycelium is the vegetative structure of fungi consisting of long filamentous hyphae, and this process of using mycelium to filter solutions has been coined mycofiltration. However, relatively little research has been done to determine whether or not mycofiltration can effectively filter out nutrients from a water source (Mnkandla et al., 2021). This study looked at oyster mushroom (Pleurotus ostreatus) mycelium and sought to determine if it is capable of absorbing nitrogen and phosphorus from contaminated water and if it is an efficient method of filtration.

The hyphae of mycelia secrete enzymes, particularly laccase and pectinase, which can be used to break down organic compounds into digestible molecules and to access nutrients (Inácio et al., 2015; Hoondal et al., 2002; Khatami et al., 2022). *P. ostreatus* is an ideal species as it produces many enzymes, grows quickly, and is tolerant to many environments (Yang, et al., 2017). Furthermore, other studies have shown *P. ostreatus* has the capacity for bioremediation of wastewater (Bhatnagar et al., 2021). This study hypothesized that the vast surface area of *P. ostreatus* and its enzymes will allow it to filter nitrogen and phosphorus from water as it passes through the colonized substrate.

The experimental design of this research was novel, but the design of the tube filtration system was inspired by a 2014 study that tested the effectiveness of mycofiltration for heavy metals in drinking water (Akpaja et al., 2014). In that experiment, researchers passed drinking water through a perforated bowl that contained Pleurotus tuberregium mycelium and used an Atomic Absorption Spectrophotometer to measure heavy metal quantities. This study used a similar experimental design to filter the solutions, but the data analysis methodology was modified to measure nitrogen and phosphorus in a novel way.

Methods and Materials

Making the Mycelium Filtration System

The setup for this experiment involved eight 8" by 2.25" PVC tubes

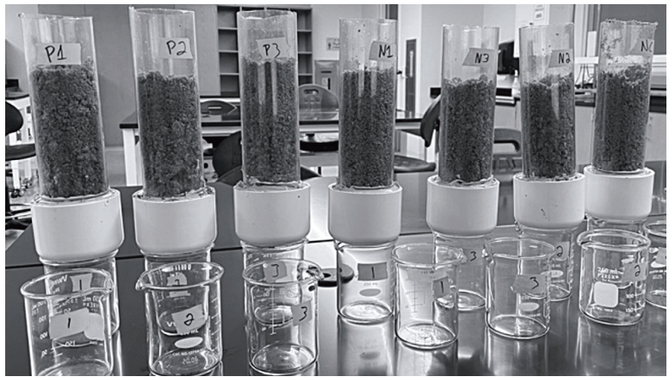

FIGURE 1

Seven of eight acrylic tubes packed with substrate. The six tubes on the left contain colonized sawdust, and the one on the right contains uncolonized sawdust as a control.

with caps on one end. Three tubes acted as a mycelial treatment for nitrogen, three as a mycelial treatment for phosphorus, and two acted as controls for each nutrient. A ¾" hole was drilled in the caps, and a wire mesh filter was placed at the bottom of each tube. Approximately one pound of *P. ostreatus* mycelium grown on sawdust (purchased from Field and Forest Co.) was loaded into six of the tubes (Figure 1). As a control, generic hardwood fuel pellets were saturated and mixed to create an uncolonized sawdust. This sawdust was then added to the two other tubes. The substrate was lightly packed into the tubes, and a small divot was created at the top of the substrate. Solutions were run through the tubes 5–10 minutes after moving the mycelium from sterile grow bags into the tubes to ensure minimal contamination.

Creating Standard Curves

To calculate nitrogen and phosphorus concentration before and after filtration, a Spectronic 200 from Thermo Fisher was used to measure absorbance. Powder capsules from the Leaf Luster Rapitest soil test kit for nitrogen and phosphorus were used as a reagent to create colorimetric assays. Isolated nutrient solutions were not available, so two nutrient mixes dense in nitrate and phosphate were used. One contained a mixture of calcium nitrate, magnesium sulfate, and potassium nitrate, and thus was missing phosphorus. The other contained a mixture of magnesium sulfate, calcium phosphate, potassium sulfate, and calcium sulfate, and thus lacked nitrogen. The nutrients were mixed with deionized water to create known concentration samples. Four samples of each nutrient mix were made at 2%, 5%, 7%, and 10% concentrations. The 7% concentration sample of each nutrient solution showed the best absorbance and was used to find the peak absorbance, which determined the best wavelength to use to measure samples. Ultimately, the absorbance of the 2%, 5%, 7%, and 10% solutions was measured at a wavelength of 390 nm for nitrogen and 1000 nm for phosphorus and used to create a standard curve for each nutrient solution.

Filtering Solutions and Collecting Data

Next, two separate liter solutions of deionized water and each nutrient mix were mixed at a 7% concentration, one liter with nitrogen and one with phosphorus. Each liter was divided into four 250 mL beakers (eight total), and a sample was taken of each of the initial nitrogen and phosphorus solutions. Then, 250 mL of each solution was poured into the respective tube and allowed

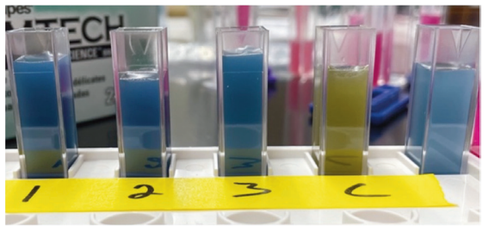

FIGURE 2

Colorimetric assay of unfiltered (far right), mycofiltered (left three), and control filtered (second from right) phosphorus solutions mixed with Luster Leaf reagent. These samples were used to determine absorbance and calculate concentration.

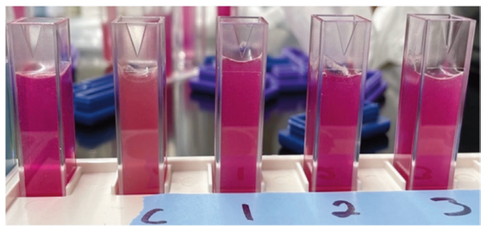

FIGURE 3

Colorimetric assay of unfiltered (far left), mycofiltered (right three), and control filtered (second from left) nitrogen solutions mixed with Luster Leaf reagent. These samples were used to determine absorbance and calculate concentration.

to drain through. Solutions took around 20–30 seconds on average to filter completely through a tube. Each solution was poured through two more times to help prolong contact with the substrate. Samples of all eight beakers were taken and mixed with the appropriate reagent from the Leaf Luster kit, and absorbance levels were measured for the initial, control, and mycofiltered solutions (Figures 2 & 3). Finally, the equations of the standard curves of known solutions were used to calculate the concentration of filtered solutions based on absorbance.

Data Analysis

The first step in analyzing the data was creating a standard curve of both the nitrogen and phosphorus known solutions (Figures 4 & 5).

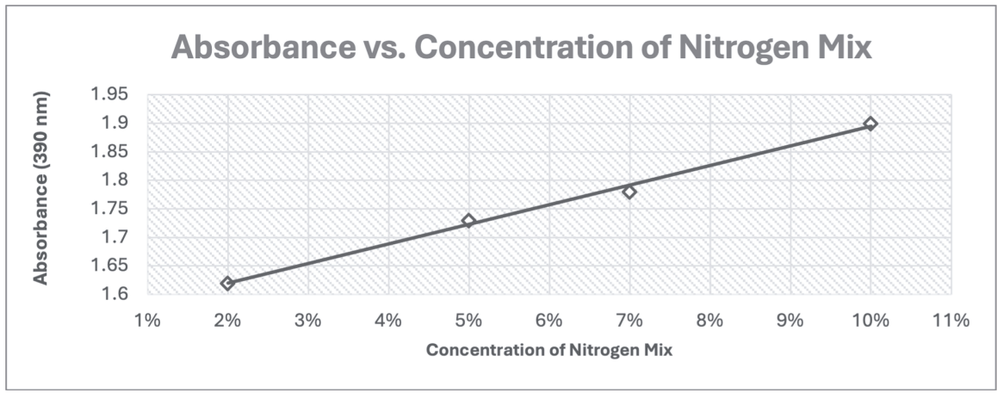

FIGURE 4

Standard curve of nitrogen solutions. This curve was calculated using the absorbances of solutions containing 2%, 5%, 7%, and 10% nitrogen nutrient mix by volume. Note that the concentration shows the percent volume of nutrient mix, not the percent of pure nitrogen. Absorbances were measured at 390 nm using spectrophotometry. The equation of the line is $y = 3.44x + 1.55$ with an R^2 value of 0.995.

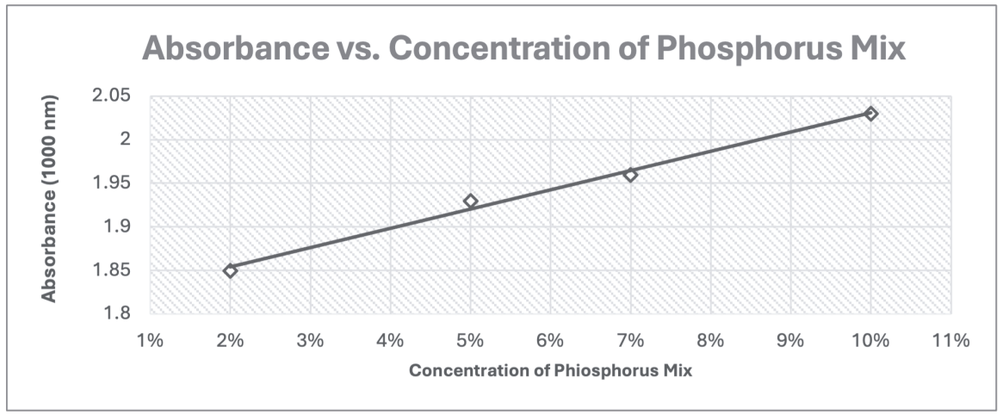

FIGURE 5

Standard curve of phosphorus solutions. This curve was calculated using the absorbances of solutions containing 2%, 5%, 7%, and 10% phosphorus nutrient mix by volume. Note that the concentration shows the percent volume of nutrient mix, not the percent of pure phosphorus. Absorbances were measured at 1000 nm using spectrophotometry. The equation of the line is $y = 2.21x + 1.81$ with an R^2 value of 0.992.

TABLE 1

Measured absorbances of each sample and the calculated concentration that was determined using the equation of the respective standard curve. The initial solutions were unfiltered samples, the controls were solutions filtered through uncolonized sawdust, and the solutions labeled 1, 2, or 3 were filtered through colonized sawdust. Nitrogen solutions were measured at 390 nm and phosphorus solutions at 1000 nm. An asterisk (*) indicates that the sample reached the Spectronic 200's max absorbance reading of 2.50.

The equations of these standard curves were then used to

Solution	Absorbance	Concentration
Nitrogen Initial	1.55	0%
Nitrogen Control*	2.50	28%
Nitrogen 1*	2.50	28%
Nitrogen 2*	2.50	28%
Nitrogen 3*	2.50	28%
Phosphorus Initial	1.97	7%
Phosphorus Control	1.88	3%
Phosphorus 1	2.07	12%
Phosphorus 2	2.07	12%
Phosphorus 3	2.02	9%

calculate the concentrations of the filtered, control, and initial (no filtration) solutions (Table 1).

Next, the means of the solutions filtered through colonized substrate were compared to the control and initial solutions in each trial (Figures 6 & 7).

Comparing the means of the filtered treatments revealed that

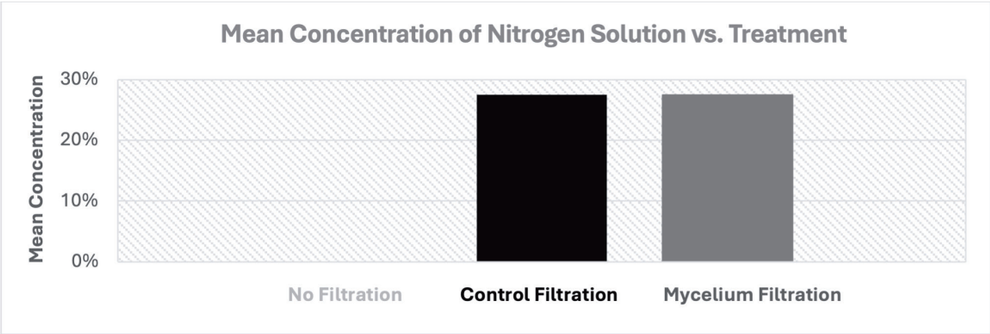

FIGURE 6

Mean concentration of nitrogen mix in solution before being filtered (left), after being filtered through colonized sawdust as a mycelial treatment (right), and after being filtered through uncolonized sawdust as a control (middle). The data shown is preliminary and not statistically significant.

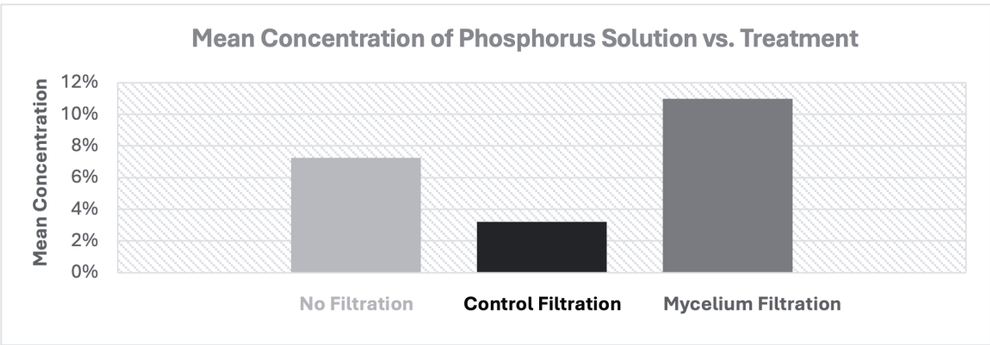

FIGURE 7

Mean concentration of phosphorus mix in solution before being filtered (left), after being filtered through colonized sawdust as a mycelial treatment (right), and after being filtered through uncolonized sawdust as a control (middle). The data shown is preliminary and not statistically significant.

the nitrogen trial was calculated at a higher concentration after both filtration treatments. However, in the phosphorus trial, the average concentration decreased in the control and increased in the mycofiltration treatment.

RESULTS & DISCUSSION

The data shows that absorbance at the selected wavelengths increased after filtering, with the exception of the phosphorus control. Furthermore, in the phosphorus trials, the solutions filtered through mycelium showed a higher nutrient concentration. Contrary to what was expected, the solutions always measured at a higher concentration of nutrients after passing through the mycelium. This suggests that *P. ostreatus* is not capable of filtering phosphorus and nitrogen with this method. However, the accurate range of the spectrophotometer used is from 0.1 to 1.0. Because of this, the data collected cannot be considered statistically significant and should be viewed as preliminary. Regardless, the data does point toward potential differences in nutrient levels between unfiltered solutions and those filtered through mycelium. These potential differences are worth exploring given more time and resources.

There are several factors that could have influenced the data and would justify re-designing aspects of the experiment and running it again to gather more conclusive results. The first major hurdle is the method of measuring nitrogen and phosphorus concentrations. At a majority of wavelengths, the solutions measured at absorbances outside the accurate range of the spectrophotometer (0.1 – 1.0). This could potentially be resolved by diluting the samples. However, without diluting, it was nearly impossible to create an accurate graph to determine the peak absorbances and choose the proper wavelength. Additionally, some of the samples reached the spectrophotometer's max absorbance of 2.5, and it is possible there was fluctuation in the solution absorbances that

could not be measured. This likely led to some inaccuracy and a lack of reliability in the measurements. For instance, the initial solution with nitrogen was mixed at a known 7% concentration, yet its absorbance registered it at 0% concentration. It would also be beneficial to use a more standard reagent. Luster Leaf does not release information on the chemicals inside their test kits, making it impossible to be certain of the chemical reaction taking place. Furthermore, using pure nitrate and phosphate as opposed to the mixture used would help limit other variables in the reaction. Were this experiment to be run again with more resources, the ideal method for measuring nitrogen would be a total Kjeldahl nitrogen analysis and inductively coupled plasma optical emission spectroscopy to measure phosphorus (Ivanov et al., 2012; Hicks et al., 2022). These methods would allow for a much more accurate measurement of the nutrient concentrations. They would also help overcome the issue of discoloration in the solutions. All of the solutions changed color when passed through the filters (Figure 8); this could have contributed to the inaccuracy of the spectrophotometer readings.

Another constraint that could have contributed to the results is the amount of time the solutions were in contact with the

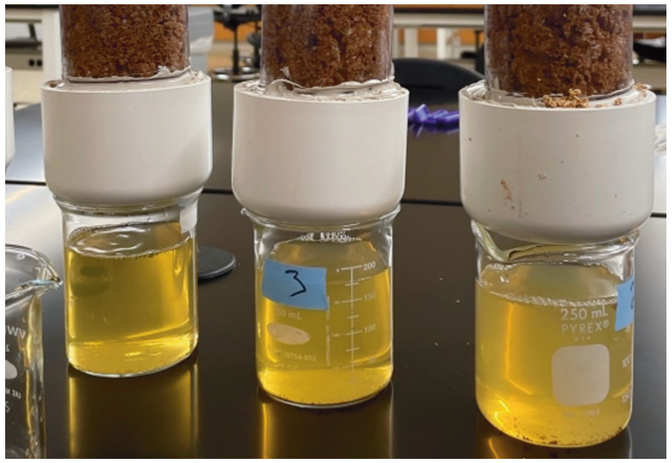

FIGURE 8

Nutrient solutions after being filtered through colonized sawdust, showing discoloration of solutions.

mycelium. The solutions were passed through the filters a total of three times. While this helped mitigate the limited contact time, the ideal setup would be to have a pump that would circulate the solution over an extended period of time or a setup where the solution soaks in the mycelium and is then drained. This would give the mycelium ample time to secrete the necessary enzymes and absorb the nutrients. This was likely a critical factor in influencing the results.

Finally, it would be ideal to grow the mycelium used in the experiment rather than purchasing it from an outside source. This would allow for consistency within the colonized substrate and control material makeup. It would also allow the substrate to be saturated with water that is already at a controlled nitrogen level. To grow mycelium on sawdust, the sawdust must be saturated with water. One potential issue with this experiment was that the substrates were saturated with a different water source. Because the control substrate was homemade and the colonized substrate was purchased, they likely had water with different nutrient concentrations to begin with. This could have led to water already in the substrate being displaced with contaminated water and resulting in inaccurate samples to test. Nutrients already in the substrate, water, or mycelium could have contributed to the fact that absorbance measurements went up after most filtrations. One potential solution to control this would be running a solution through the substrate until no nitrogen or phosphorus can be detected. This would act as a wash and ensure there is no nitrogen or phosphorus in the tubes to begin with. Growing the mycelium in house and controlling the water source used to saturate the substrate would also eliminate this problem. It would also allow the levels of nitrogen in the sawdust to be controlled, ensuring that none leaches from the substrate. Furthermore, growing the mycelium in the tubes would eliminate the issue of disturbance. Mycelium is a living organism, and transferring it from the grow bags to the

acrylic tubes could have disturbed the mycelium and lowered its enzyme productivity. Growing the mycelium in the tubes would ensure the mycelium is undisturbed and that it is at high enzyme productivity levels.

The results from this study were inconclusive, but with a few adjustments in methodology, this experiment could contribute valuable information to the scientific community and help combat the issue of eutrophication in the Chesapeake and watersheds around the world. Given more time and resources, the three most important modifications to make would be enhancing the method of measuring nutrient concentration, filtering the water for a prolonged period of time, and growing the mycelium in house. By making these modifications, researchers could provide far more conclusive results on whether or not this is a viable option for filtering runoff. Ultimately, this is an exciting technology with broad-reaching applications that is worthy of further exploration and research.

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