Microplastic interference influences *Pseudomonas fluorescens* in denitrification efficiency of wastewater treatment

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Note: *indicates figure created by student researcher

Abstract

Wastewater treatment plants (WWTP) play an essential role in pathogen and contaminant removal in wastewater. While developed countries treat approximately 70% of industrial wastewater prior to discharge, only about 8% is treated in developing countries. WWTP solutions can reduce the solids load, including microplastics, by up to 98.4%. Still, it is estimated that about 65 million microplastics are released into aquatic environments every day. Microplastics that are 20-300 µm in size are additionally considered extremely difficult to remediate and may be problematic for the denitrifying gram-negative bacteria *Pseudomonas fluorescens*, which is capable of polyethylene degradation. In order to explore this interaction, four bioreactors were established that contained 0% (control), 0.1%, 0.25%, and 0.5% plastic waste by weight, and inoculated with a *Pseudomonas fluorescens* culture. In the next five days, nitrate, nitrite, and polyethylene degradation were monitored through two trials. The 0.5% group saw the slowest nitrate reduction, indicating that the higher concentration of microplastics were inhibiting denitrification. The nitrate concentrations observed at the 0.1% group were most similar to the control. All groups had decreasing amounts of microplastics present, thus indicating some degree of biodegradation. This study suggests that microplastics impede *Pseudomonas* mediated denitrification while simultaneously being biodegraded. Future studies should look at different types of microplastics, lengths of time, and using different bacterial strains for the same purpose.

Keywords: Microplastics, *Pseudomonas fluorescens,* Denitification, Wastewater, Ecology, Environmental Science, Climate Science

1. Introduction

Wastewater treatment plants (WWTPs) have gained attention as key facilities for managing water resources due to growing global interest in environmental sustainability and water quality. Although these plants are intended to eliminate pollutants and pathogens from wastewater (Petrovic et al., 2013), concerning data indicates that more than 80% of wastewater worldwide is returned to the environment untreated (United Nations Environment Programme 2019). The need for better wastewater management techniques is highlighted by the difference in treatment rates between developed and developing nations. Only 8% of industrial wastewater in developing nations is treated, compared to roughly 70% in the developed world (UNESCO, 2017). The existence of microplastics exacerbates this, as they have significant impacts on aquatic life and human health when they penetrate the food chain (Carr et al., 2016).

Understanding how plastic influences the nitrogen cycle in wastewater treatment is becoming more crucial given the rising frequency of plastic waste in our surroundings. By means of this information, treatment procedures can be improved, environmental protection can be strengthened, and finally better nitrogen and plastic pollution management results. Millions of microplastics are released daily even if WWTPs can remove a considerable amount of microplastics since the volume of water being treated is great (Murphy et al., 2016). Significant in lowering the nitrogen content in treated wastewater is denitrification, the biological conversion of nitrate NO_3, into nitrogen gas N_2, by anaerobic microbial action (Stamper & Semmen, 2012).

May denitrification be ineffective, nitrogen levels in oceans would rise exponentially, causing nutrient pollution and devastating algal blooms upsetting aquatic ecosystems (Zhou et al., 2023). My study aims to offer insightful analysis of the elements affecting denitrification, especially in relation to the presence of microplastics, and could result in better treatment methods reducing nitrogen removal and lessening the effects of plastic pollution. The foundation for examining the complexity of wastewater treatment, the environmental problems it generates, and the creative ideas required to reach ideal water quality and sustainability is this introduction.

2. Literature Review

2.1 *Definition and Environmental Impact*

Microplastics are small plastic particles that are less than 5mm in size (Andrady, 2011). Microplastics environmentally accumulate which poses risks to aquatic life (Carr et al., 2016).

2.2 *Sources and Types of Microplastics*

Microplastics (MPs) in WWTPs mostly originate from personal care and cosmetic products (PCCPs), according to Yaseen and colleagues' "A global review of microplastics in wastewater treatment plants: Understanding their occurrence, fate and impact" (Yaseen et al., 2022). Polyethylene (PE) derivatives are a major contributor. PE accounts for 22% of the identified MPs (Yaseen et al., 2022), according to the study, which revealed that microfibers (57%) and fragments (47%), are the most common forms of MPs in both influents and effluent of WWTPs.

2.3 *Removal Efficiency of WWTPs*

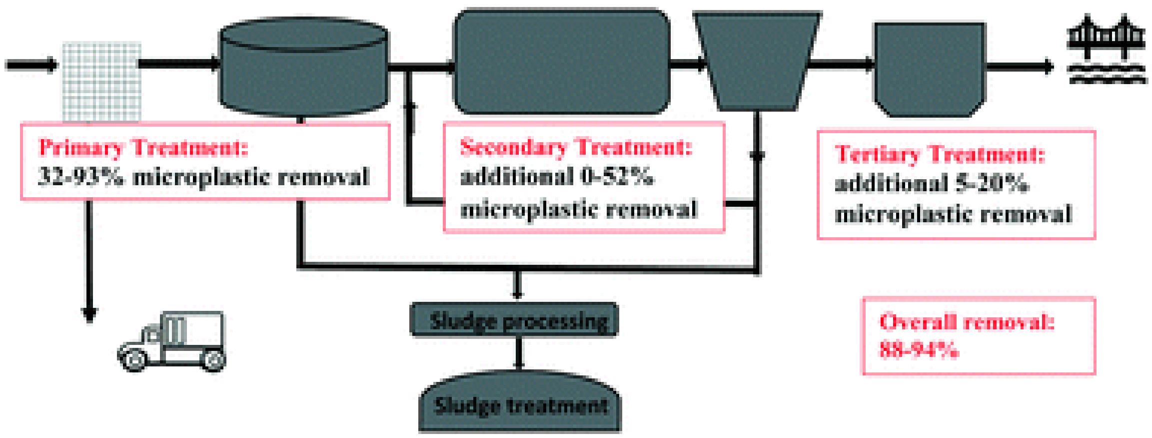

Figure 1. WWTP microplastic removal (Iyare et al., 2020)

WWTPs can remove up to 98.41% of microplastics from wastewater, but due to the high volume of treated water, a significant number still enters aquatic environments (Murphy, et al. 2016). Paul Iyare along with other researchers wrote an article, "Microplastics removal in wastewater treatment plants: a critical review" and their aim was to examine and quantify the removal efficacy of microplastics by WWTPs. Microplastic removal rates in 21 studies were compared in this critical review. Secondary and tertiary WWTPs removed an average of 88% and 94% of microplastics, respectively (Iyare et al., 2020). Despite this, WWTPs release an estimated 65 million microplastics daily, highlighting the need for improved treatment technologies and management practices (Murphy et al., 2016). A study done by Sadia and others called, "Microplastics pollution from wastewater treatment plants: A critical review on challenges, detection, sustainable removal techniques and circular economy" show that specifically microplastics of 20-300µm in size are problematic for removal in WWTPs (Sadia et al., 2022).

2.4 *The Nitrogen Cycle and Environmental Consequences*

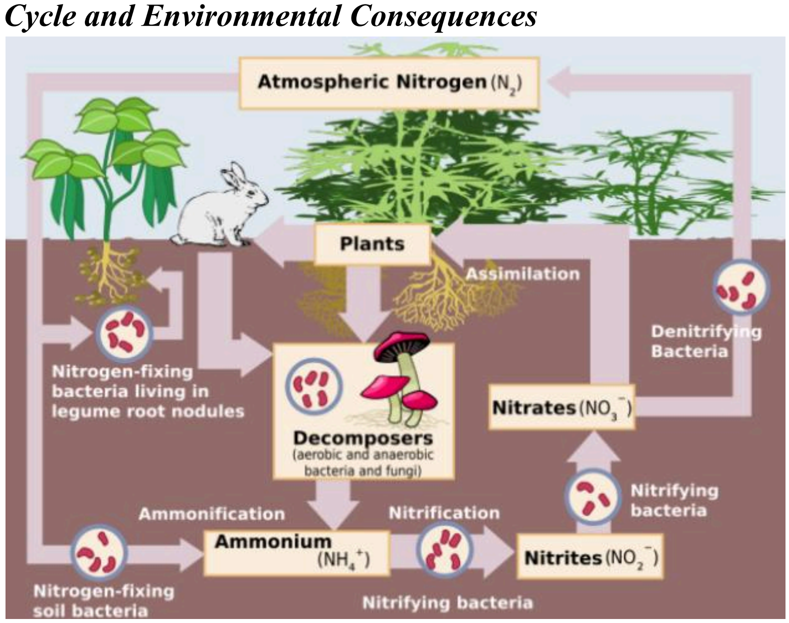

Figure 2. Nitrogen Cycle (UNDERSTANDING GLOBAL CHANGE, 2025)

Denitrification is used globally in biological removal of nitrogen from wastewater to enhance water quality (Lu et al., 2014). Waterborne excess nitrogen could lead to eutrophication, causing toxic algal blooms, reducing oxygen levels, and killing aquatic life.

2.5 *Microbial Approaches to Nitrogen Removal*

Pseudomonas fluorescens is a denitrifying gram negative, rod shaped bacterium (D'Souza et al., 2023). *P. fluorescens* is a heterogeneous bacterial population that is found in many environments, such as soil, water, and industrial settings (Radhakrishnan & Ananthasubramanian, 2012). In the article, "Characterization and metabolic pathway of *Pseudomonas fluorescens* 2P24 for highly efficient ammonium and nitrate removal" by Huang and colleagues, the ability of a *P. fluorescens* strain 2P24 to remove nitrogen was screened in synthetic and real wastewater. The results revealed that when ammonium, nitrate, or nitrite served as the sole source of nitrogen, strain 2024 removed 96.63% of ammonium and 100% of nitrate and nitrite within a period of 48 hours (Huang et al., 2023). This indicates an encouraging method to remove nitrogen from wastewater.

2.6 *Microbial Action on Plastics*

Furthermore, studies by Mandal and colleagues show that some microbes have developed genes that influence biotic and abiotic factors that affect changes in plastic pollutants (Mandal et al., 2024). By using the pollutants' corresponding carbon content as development energy sources, they eradicate microplastics (Mandal et al., 2024). The presence of these bacteria can accelerate the degradation process, making wastewater treatment more efficient and ecologically friendly. This is especially advantageous because wastewater treatment facilities are primarily concerned with eliminating microplastics. This dual ability of *P. fluorescens* promotes healthier aquatic environments by reducing microplastics and addressing nitrogen pollution.

2.7 *Polyethylene Degradation by Pseudomonas species*

Building on this, research by Tamnou et al. in "Biodegradation of polyethylene by the bacterium *Pseudomonas aeruginosa* in acidic aquatic microcosm and effect of the environmental temperature" demonstrates that the bacterium *Pseudomonas aeruginosa* can effectively degrade polyethylene in aquatic environments at a neutral pH of 7 and temperatures ranging from 30-37 °C. This connection is crucial as it suggests that the presence of *Pseudomonas* species in WWTPs could facilitate the biodegradation of polyethylene microplastics, particularly given the environmental conditions often found in these treatment facilities. However, it is important to note that the biodegradation process of polyethylene by *P. fluorescens* under various environmental conditions, along with the impact of the degradation products on microbial cells, remains incompletely understood.

3. Research Gap

The goal of this study is to close the substantial knowledge gap regarding the degradation of polyethylene by *P. fluorescens* under various environmental conditions. The impact of microplastics on *P. fluorescens* and the degree to which microbial cells are impacted by the degradation products are still poorly understood. It's unclear if microplastics will affect this bacterium's capacity for denitrification. Not much research has been done on this, particularly under extremely specific circumstances. This knowledge gap presents an excellent opportunity for further investigation into the optimization of *P. fluorescens* to optimize the removal of microplastics from wastewater.

This study has the overall aim of answering the following research question through the analysis of the relationship between microplastics and the denitrification process: How does the presence of microplastics influence the efficiency of denitrification abilities of *Pseudomonas fluorescens* in wastewater treatment plants? It is important to reduce the facts of microplastics and high nitrogen in water for a number of reasons. As microplastics are consumed by aquatic life and inflict damage on their health and, by extension, the health of the entire food chain, they represent a serious threat to aquatic ecosystems. Aquatic organisms' bodies might accumulate such tiny particles, possibly passing the toxins to humans and other higher trophic levels. Furthermore, excess nitrogen in waste bodies degrades water quality.

In addition to harming biodiversity, this also contaminates recreational spaces and drinking water sources. We can safeguard aquatic ecosystems, conserve biodiversity, and guarantee that water resources are sustainable for future generations by eradicating microplastics and controlling nitrogen levels. The development of efficient methods to treat wastewater and lessen these environmental problems depends on this study on *P. fluorescens*' capacity to reduce excess nitrogen in relation to microplastics.

4. Experimental Design and Methodology

4.1 *Research Hypotheses*

This study explores how microplastic interference influences *Pseudomonas fluorescens* in denitrification efficiency of wastewater treatment. In deciding what methodological approach best fits this study, the following hypotheses were made to guide the methods in alignment with the research question:

1. Polyethylene microplastics will decrease the efficiency of the denitrification abilities of *P. fluorescens.*
2. *P. fluorescens* will degrade the polyethylene in the experimental groups.

4.2 *Materials*

All Chemical compounds were bought from Carolina Biological Supply. The denitrifying bacteria, *P. fluorescens*, with a biosafety level (BSL) of 1 was provided by Carolina Biological Supply. This specific bacteria was used for this experiment because it is found in wastewater treatment plants (Radhakrishnan & Ananthasubramanian, 2012) and not many studies focus on this bacteria, showing novelty and adding new research. Safety Data Sheet (SDS) was reviewed with an adult sponsor. I had adult supervision at all times and wore gloves and goggles when handling bacteria and chemicals. Disposals of bacteria and chemicals were handled in accordance with local, state, and national guidelines.

4.3 *Experimental Design*

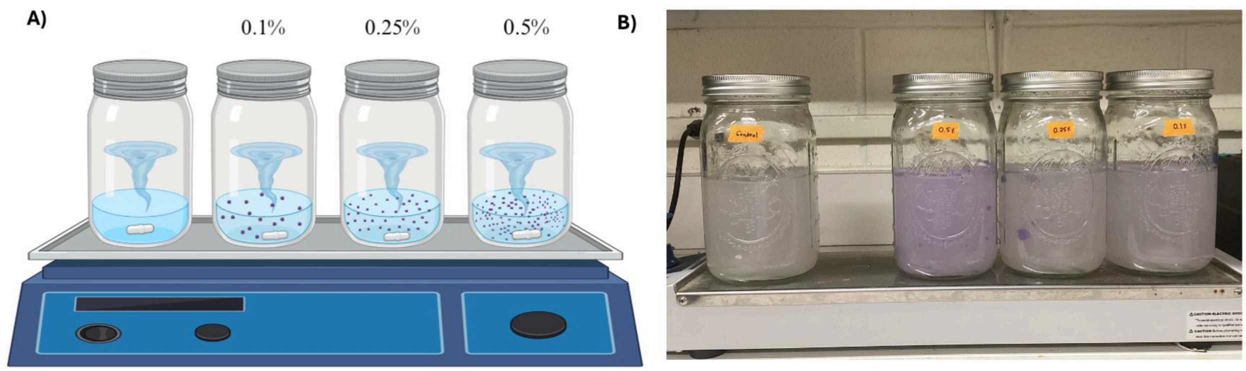

Figure 3. Control and Experimental Groups* A) Experimental Design B) Lab Setup

I created four treatment systems (bioreactors) that simulate wastewater treatment. Each system was set up with the same volume of wastewater (500mL) in mason jars with controlled conditions (temperature and pH). Every reactor had a stir bar and was on a magnetic stirrer that was continuously spinning. All reactors were inoculated with *P. fluorescens*, which is known for its denitrifying capabilities (D'Souza et al., 2023). This ensured that any differences observed in nitrogen transformation were due to the plastic concentration. The experimental groups received MP, specifically polyethylene. As stated before in the literature review, polyethylene accounts for 22% of the detected MPs (Yaseen et al., 2022) and is one of the most prominent MP found in WWTPs, which is why it was used in this study. Also stated before, due to the fact that MPs that are 20-300µm in size are problematic for removal in WWTPs (Sadia et al., 2022) polyethylene 180-212µm in size were used for this experiment. This was done because I wanted to stimulate real world conditions to see how these plastics affect treatment processes and microbial communities. This helps highlight the challenges WWTPs face with plastic pollution. The setup of the reactors with treatment systems containing 0% (control), 0.1%, 0.25%, and 0.5% plastic waste by grams is designed to evaluate the impact of varying concentrations of MPs on the wastewater treatment process. Given that MPs in WWTPs are primarily derived from personal care and cosmetic products — which often consist of polyethylene derivatives, it's essential to understand how these different concentrations can influence the efficiency of microbial activity and denitrification. Through the use of a control group that has 0% plastic waste, this establishes a baseline from which to compare with the remaining treatment systems. The small plastic waste additions (0.1%, 0.25%, and 0.5%) help to determine the impact of microplastics on the ability of microbes to clean wastewater. This is especially important because studies have determined that microfibers and fragments are common types of microplastics in wastewater discharging and incoming wastewater treatment plants (Yaseen et al., 2022). The selection of 0.1%, 0.25%, and 0.5% of plastic waste quantities is crucial since WWTPs treat many plastics. Given that the quantity is 500 mL of wastewater, incorporating lower percentages of microplastics assists in regulating the quantity of plastic waste being added to ensure that the rates remain realistic and do not hamper the treatment process. This process is beneficial, especially when analyzing results to more clearly see how different levels of plastic waste affect the small organisms and how effective the treatment is, without adding too much plastic that could skew the result.

4.4 Preparation and Analysis

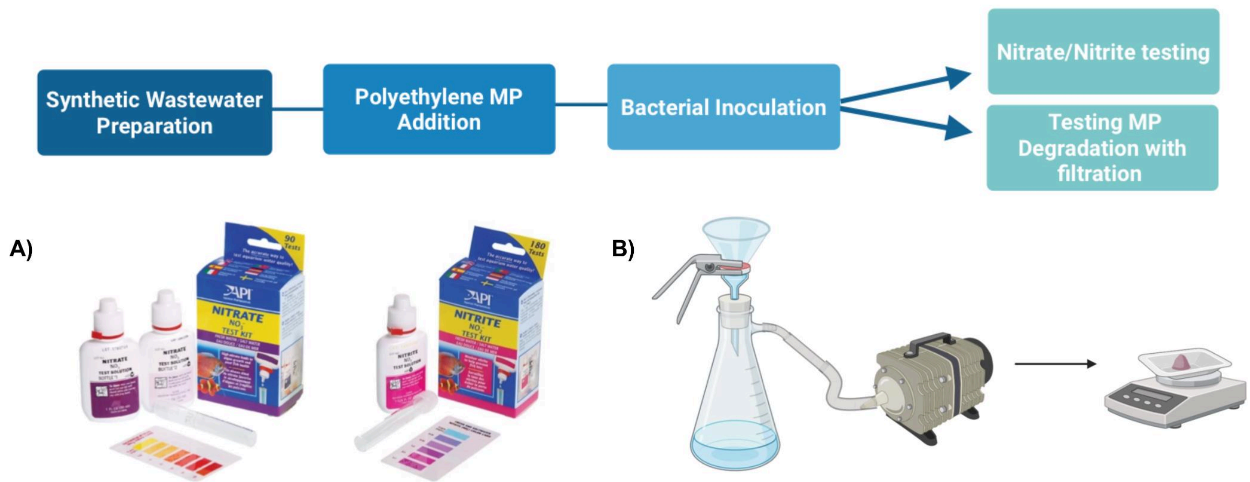

Figure 4. Preparation of experiment and testing* A) Nitrate/Nitrite testing B) MP filtration then dry massing

To prepare the synthetic wastewater, I created a 1.0L stock volume media twice. Then I put the created synthetic wastewater into the mason jars with a volume of 500mL each. Polyethylene was massed on a microscale for accuracy, then added to the mason jars. The mason jars were autoclaved at 121 degrees for 40 minutes to ensure everything is sterile (standard sterile technique). Initially, when I got my bacteria in vials I transferred them onto plates, then to inoculate the jars I swabbed the plates and put the bacteria into the reactor.

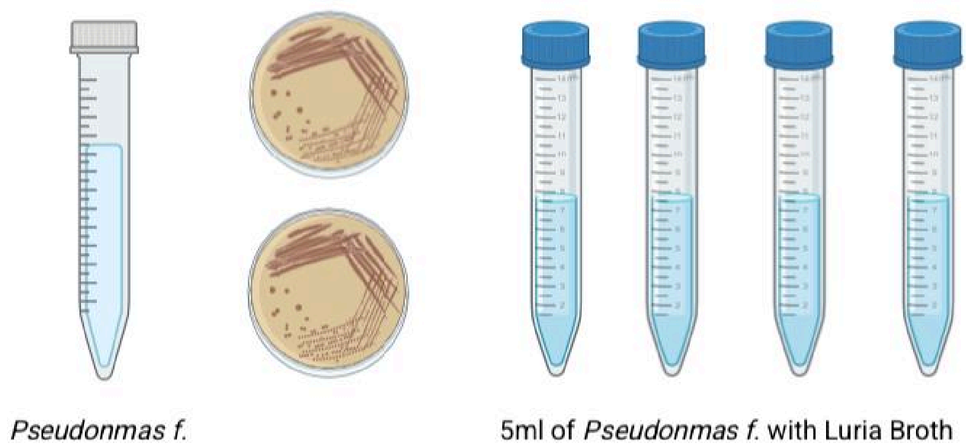

Figure 5. Adding *P. fluorescens to Luria Broth*

However, my methods were changed to adding swabs of the bacteria into 5mL of Luria Broth (LB) to help support the growth of *P. fluorescens* in the jars due to complications. Changing the method to insert swabs of bacteria into 5 mL of LB helps *P. fluorescens* grow better by creating a nutrient-rich environment that helps bacteria multiply (Savijoki et al., 2022). Luria Broth is specially formulated to help most bacteria grow, including *P. fluorescens*, by providing essential nutrients such as amino acids, vitamins, and salts (Choi et al., 2022). Having LB guarantees the bacteria can grow optimally (Savijoki et al., 2022). This is especially important if there had been a problem at the start that could have inhibited their growth. This method guarantees that *P.*

fluorescens grows better in the experiment, giving us more valid and informative results on how they interact with microplastics in wastewater treatment.

To test for nitrate and nitrite I used API testing kits (Figure 4). In order to test for MP degradation, I created a vacuum filtration pump to help speed the process of the filtration (Figure 4). After the filter paper was dry, I was able to collect all the MP and dry mass the MP on a microscale.

5. Results

The procedure for the API kits to test nitrate was to draw a 5 mL sample from each far and it into the sample testing container. Following the protocol in the API nitrate test kit, 10 drops of Bottle #1 were added to the sample, then 10 drops of Bottle #2, shaking for two minutes after the 10 drops. For about five minutes, the mixture was let to sit in order to facilitate the reaction, the color would change. The color of the sample was then compared with the color chart enclosed with the kit in order to identify the concentration of nitrate. The concentration of nitrate was then noted according to the comparison of color and any other instructions provided for safe disposal of the sample.

In order to test for nitrite using API kits, a 5 mL water sample must be taken from each jar and placed in the sample testing container. Five drops of the test solution should be used, as directed in the API nitrate testing kit. For about five minutes, the mixture was let to sit in order to speed up the reaction. To determine the nitrite concentration, the color of the sample was then compared to the color chart that came with the kit. Lastly, the color comparison was used to determine the nitrite level, and any further guidelines for the sample's appropriate disposal were followed.

5.1 *Nitrate Pre-Trial Data*

Day's of Experimentation	0g	0.1g	0.25g	0.5g
1	18	18	18	18
2	18	18	18	18
3	18	18	18	18
4	18	18	18	18
5	18	18	18	18

Figure 6. Nitrate Pre-Trial Data
This chart shows the pre-trial data for nitrate concentration in the span of five days. The nitrate concentrations stayed the same in the control and experimental groups.

5.2 *Pre-Trial Microscale WWTPs*

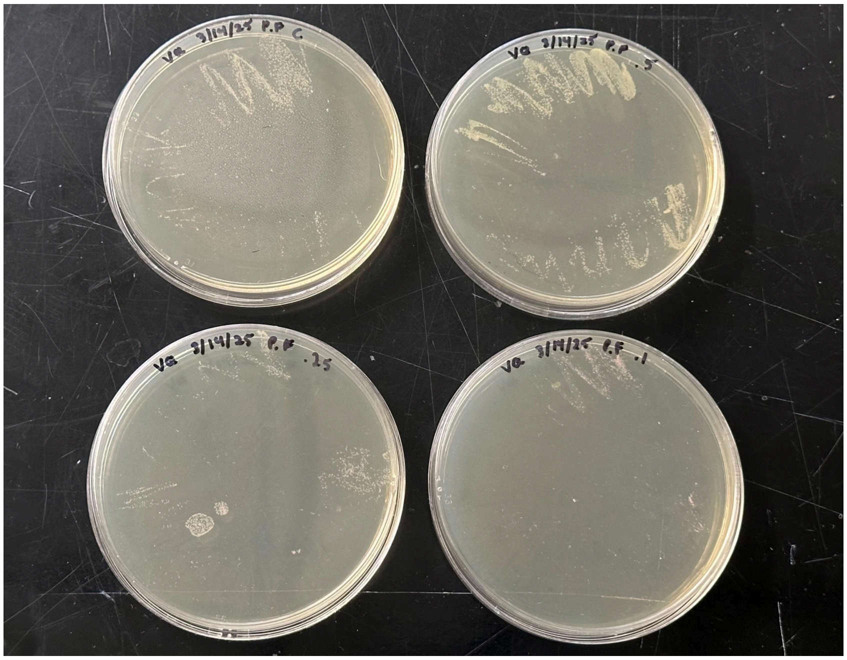

Figure 7. Pre-Trial Microscale WWTPs
After the pre-trial ended I made plates to see if there was any bacteria growing in the jars because the results did not show a reduction or increase in the nitrate concentration. There is little to none *P. fluorescens* on the plates which shows the bacteria was not supported. This led me to make a change in my methods for *P. fluorescens* to be able to grow in the jars.

5.3 *Nitrate Data*

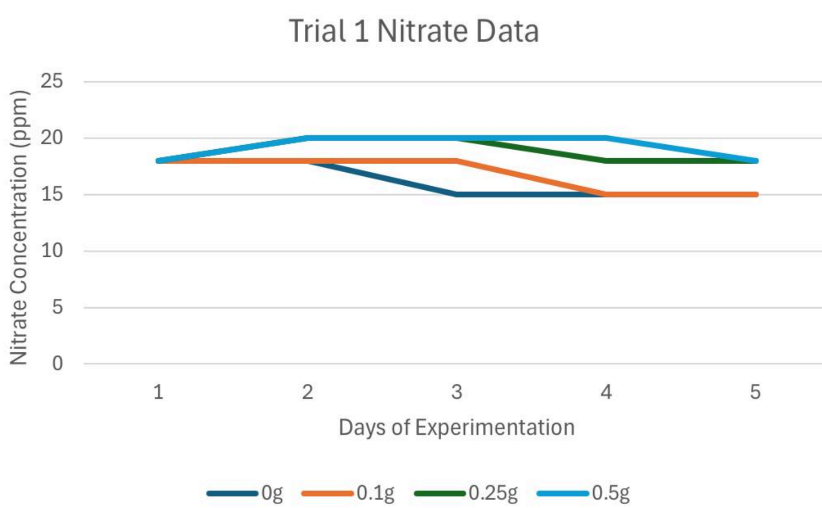

Figure 8. The nitrate concentration of control and experiment groups in the time span of five days for trial 1.

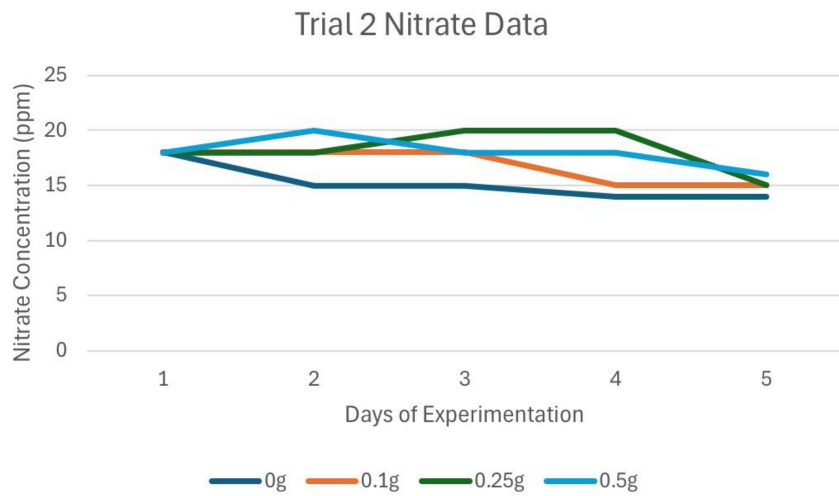

Figure 9. The nitrate concentration of control and experiment groups in the time span of five days for trial 2.

There were two trials conducted within the span of five days. 0.5g showed the slowest reduction and least reduction in nitrate concentration, this was shown in both trials. This demonstrates that microplastics were potentially inhibiting *P. fluorescens* denitrification abilities. 0.1g was the most similar to the control and also showed similar results to the control, shown in both trials.

5.4 ***Nitrite Data***

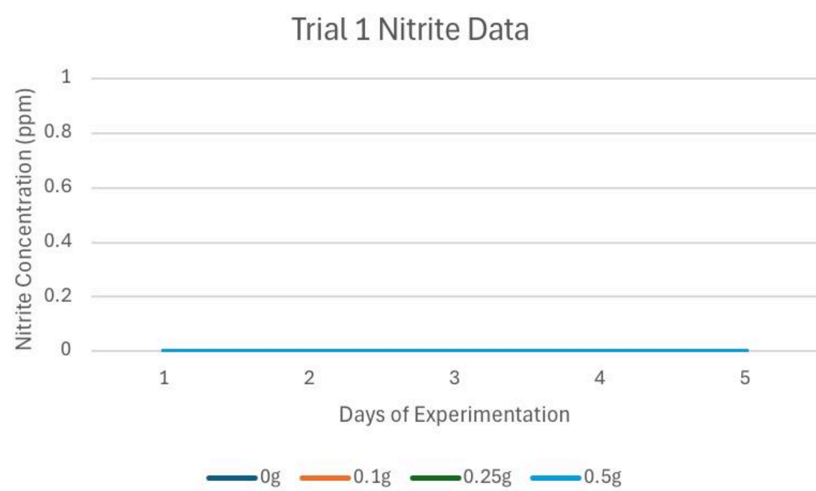

Figure 10. The nitrite concentration of control and experiment groups in the time span of five days for trial 1.

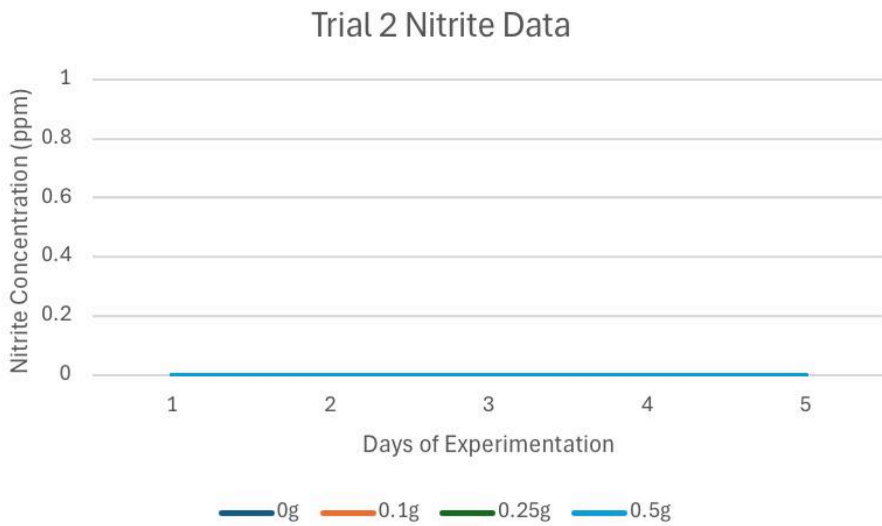

Figure 11. The nitrite concentration of control and experiment groups in the time span of five days for trial 2.

The nitrite concentration was tested simultaneously with nitrate data, the nitrite stayed at zero within the span of five days for both trials. Nitrite concentration was tested to ensure that only denitrification was happening and not any other processes.

5.5 ***Microscale WWTPs***

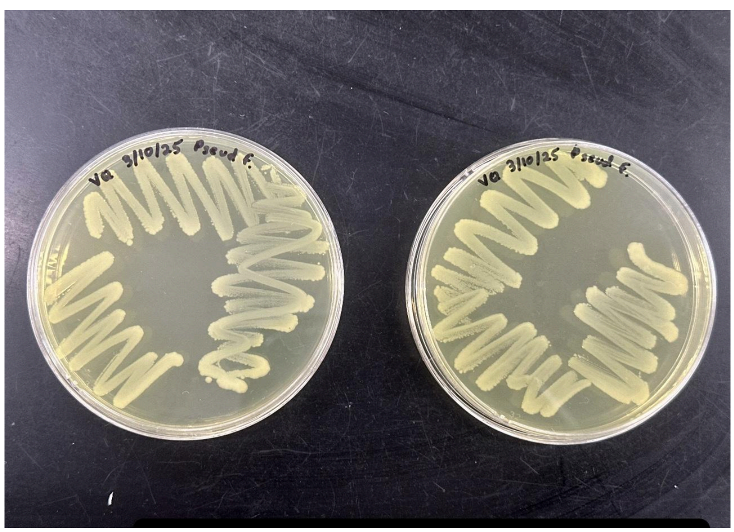

Figure 12. Plates of bacteria after experiment.

After the trials I made plates to see if there was any bacteria growing in the jars. These plates indicate that bacteria were effectively supported in a similar environment to a WWTP. This proves that denitrification processes were expected to occur within these environments and were observed in all groups.

5.6 ***Polyethylene Degradation***

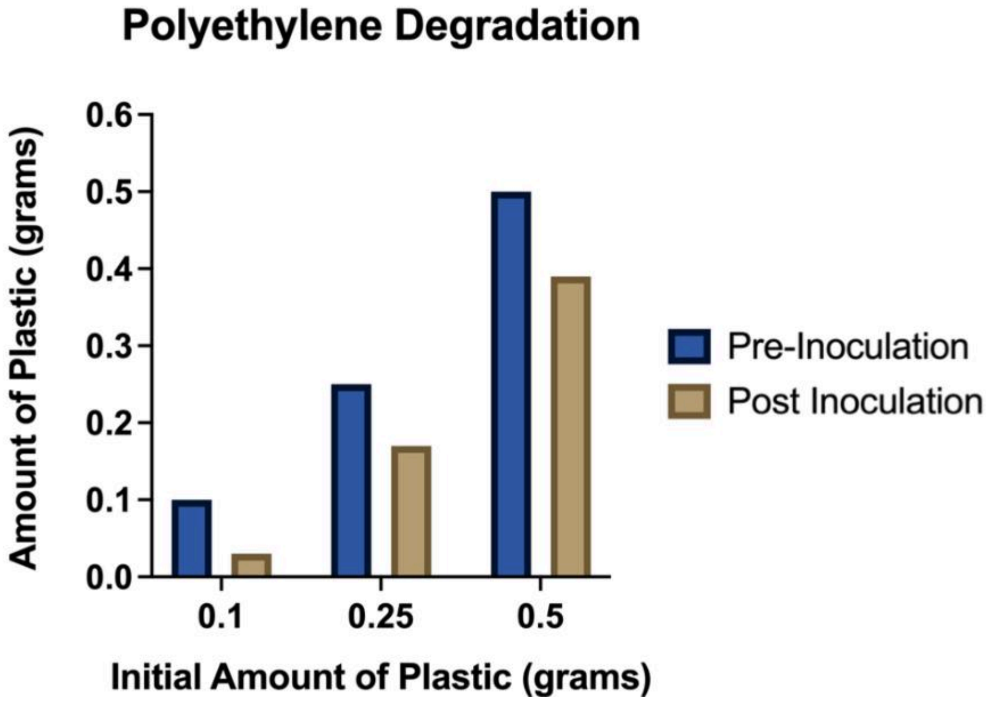

Figure 13. The amount of plastic (grams) before and after bacterial incoluation.

After the experiment ended, I tested to see if the amount of microplastics decreased. The post-inoculation in all experimental groups, shows a decrease in microplastics. This supports the hypothesis that *P. fluorescens.* was effectively degrading the microplastics.

6. Discussions

My research shows the impact of microplastics on denitrification under the concentrations evaluated. That the group with the highest plastic concentration (0.5g) recorded the slowest nitrate reduction, indicates a good inhibition of the metabolism of the denitrifying bacteria. This is because denitrification is a crucial process in the nitrogen cycle which consumes nitrates and reduces it to nitrogen gas and plays a key role in aquatic systems' ecological balance. The inhibition we witness at high concentrations ask questions about how plastic pollution could disrupt the denitrification process to lead to the accumulation of nitrates in the environment to cause eutrophication and other unfavorable ecological effects.

The low microplastics concentration (0.1g) treatment group also demonstrated a nitrate concentration response similar to the control treatment group, and it indicates that low concentrations of microplastics could not greatly inhibit denitrification. This has particular relevance to domain conclusions as it further indicates that microplastic effects on other microbial processes could also be concentration-dependent. Understanding of the concentration relationship will be vital in environmental monitoring and management as perhaps demonstrates that there are threshold concentrations of microplastic releases below which denitrification is not affected, permitting sustained ecosystem functioning. Such would be beneficial knowledge to instigate policy for plastic waste enhancement in water systems.

The fact that nitrite was not detected across both trials bolsters the conclusion that nitrification was not taking place is an integral part of understanding the nitrogen cycle. By focusing on denitrification alone we determined that the bacteria were effectively converting nitrate into nitrogen gas with the in between processes of nitrification never taking place. This is significant because it implicates *P. fluorescens*, as successfully facilitating denitrification utilizing the experimental parameters. The observations of plates with no contamination lends some credence to the conclusion that the experiments prepared the appropriate environmental conditions and that it was comparable to environments found in wastewater treatment plants (WWTPs).This finding is encouraging, as it suggests that *P. fluorescens* could be harnessed in bioremediation efforts to manage both nitrogen levels and microplastic pollution.

The reduction of microplastics in every experimental group indicated that *Pseudomonas fluorescens* not only carried out denitrification, but was also acting on microplastic degradation. The importance is particularly evident when choosing specific microbes to enact bioremediation strategies where nitrogen pollution and plastic waste remediation might resolve overlapping issues. My results may also have broader implications for environmental policy and management, and future foundational research on microbial communities and microplastics in ecosystem sustainability.

While my research may only have addressed a small part of the complexity that exists ecologically between microplastics and microorganisms, I nonetheless found how microplastic concentration shaped denitrification — aligning to emphasize how differing levels of plastic pollution reduce or promote nitrogen cycling, and overall ecosystem health. Therefore, my research may provide initial perspectives for future research and management decisions that aim to correct plastic pollution, educate on nutrient loading practices, and set up resilience mechanisms for aquatic ecosystems that are otherwise under pollution stressors. The likelihood of *P. fluorescens* acting as a bioremediator provides paths to investigation to reduce both nutrient loading and plastic, without the risks associated with biomanagement approaches typically taken.

7. Limitations

One limitation of my research was the availability of HACH nitrate and nitrite testing samples. The availability of testing samples impacts the accuracy of both High Range and Low Range nitrate and nitrite measurements. Collecting accurate data is challenging without testing samples and is essential for making acceptable inferences while conducting experimental work. Imperfect measurements may lead to misinterpreting how polyethylene microplastics affect the ability of *P. fluorescens* to act denitrifying and, in turn, threaten the credibility of my research.

Furthermore, another area of limited investigation was the lack of availability regarding a nitrogen gas (N_2) machine, which would have allowed me to test for the gas version of nitrogen. Having access to measure the gas form is important when you measure denitrification because the gas form of denitrification is the ultimate end-point in the metabolic process that occurs during denitrification. Since I was not able to measure N_2 levels, I miss characterizing the denitrification process and therefore do not know to what extent *P. fluorescens* was able to convert these nitrogen compounds into a non-toxic level gas form.

During the pre-trial preparation, there was some trouble with adding bacteria to my reactors. The addition of bacteria did not allow me to conduct one of my planned trials which complicated the experimental plan. I had to make adjustments to my original methods in order to accommodate this issue. I placed the denitrifying bacteria in 5 mL of LB broth. The presence of

LB broth serves as a better starting environment for the bacteria and promotes bacterial growth. LB broth is rich in nutrients that promote bacterial growth and allows for cultured bacteria to be viable and active for your next set of experiments. I needed to modify my methods to accommodate the previous mistake and to ensure my trials could still yield results based on the limitations you had placed on bacteria during the pre-trial preparation.

8. Implications and Future Research

Future research on microplastics and the impacts of microplastics on ecosystems should continue to broaden the types of microplastics used and the types of bacteria used. The numerous types of microplastics that exist are diverse and combined by physical properties (e.g., surface properties, size) as they can also be defined by chemical identity. With a wider variety of microplastic types, the research from these studies and identified bacteria could lead to a more comprehensive understanding of both the interactions of microplastics in the environment and the effects of microplastics on biotic communities. In particular, the number and types of microplastics could affect the degree of bacterial colonization, which would affect how nutrients cycle, biodegradation potential, and other functions that contribute to ecosystem health.

Additionally, the type of bacteria used is of consequence as well. Because they have different metabolic pathways, different bacteria would have different behaviors and responses to the different microplastics used in studies. Understanding those interactions would provide a better understanding of the potential for particular bacteria to be useful for degrading or transforming microplastics and would enhance knowledge of the potential for certain bacteria to be used in bioremediation. For example, some bacteria can metabolize specific plastic polymers (single plastic species) and/or, some bacteria only colonize the plastic without degrading it. Thus, assessing and looking at responses of a range of bacteria to a range of microplastics, would allow researchers to identify potential bioremediation candidates.

Denitrification, in addition to microplastics, is also an important microbial process that transforms additional nitrogen compounds, such as nitrate into nitrogen gas and releases it to the atmosphere. Denitrification can serve to lessen the negative impacts of nitrogen pollution from wastewater treatment plants that create eutrophication in aquatic ecosystems. The application of the denitrifying bacteria (*Pseudomonas* species, *Paracoccus* species, and *Bacillus* species) in wastewater treatment, can also increase nitrogen removal efficiencies from wastewater prior to discharge into the ocean. By denitrifying nitrate to nitrogen gas, denitrification can remove nitrogen that could contribute to nutrient loading in marine ecosystems. During the assessment of denitrifying bacteria, we consider the optimal oxygen gradients and organic carbon for denitrification, thus improving operational efficiencies and striving for the depletion of the nitrogen compound that could result in harmful nitrogen pollution. Thus, in addressing the environmental and health impacts of microplastics, potentially minimize loading of nitrogen pollution, and improve denitrification efficiencies, we could restore aquatic ecosystems. This is a meaningful and necessary step to progress in finding solutions for dealing with microplastics, and nitrogen pollution.

9. Fulfillment of the Research Gap and Conclusion

Microplastics, more specifically polyethylene, are ubiquitous pollutants that have the potential to have some serious effects on microbial processes in wastewater treatment plants. The question posed in this study, "How does the presence of microplastics influence the efficiency of denitrification abilities of *Pseudomonas fluorescens* in wastewater treatment plants?", is an important knowledge gap in environmental microbiology to address. However, the study can also highlight how microplastics interact with the denitrification process of *P. fluorescens*, and in turn help build the knowledge of the double whammy of plastics pollution and nitrogen regulation in aquatic environments.

The study provides evidence of the hypothesis that the presence of the polyethylene microplastics was going to decrease *P. fluorescens*' denitrification efficiencies, and therefore implicate that the microplastics may be inhibiting these bacteria's metabolic processes and leading to less efficient nitrogen removal. This has significance due to nitrogen pollution resulting in eutrophication and death of organisms both directly and indirectly, and must be balanced via efficient denitrification. Thus, it signifies that microplastics have adverse effects on wastewater treatment, but also highlights the impetus to innovate solutions to decrease plastic waste and nutrient nutrient pollution. That said, *P. fluorescens*' biodegradation of polyethylene was an interesting aspect to my analysis.

My research indicates that microplastics can be amended via biodegradation by bacteria. This is important since it exposes a new avenue for bioremediation of polluted areas. This was definitely an interesting dual purpose for preventing pollution of microplastics and carrying out critical denitrification, and new possibilities arise regarding innovative wastewater treatment alternatives to improve pollutant removal and ecosystem functions. Furthermore, the creation of sustainable systems that manage plastic pollution, and help create clean water quality that is essential for the sustainability of aquatic ecosystems around the world, and human health, could also be applied as a result of having carried out this research.

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