

**The Effect of Pseudomonas on Removing Nitrates in Wastewater.**

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**Abstract**

Nitrates can be harmful to humans but especially harmful plants and ecosystems. Nitrates can be found in fertilizer, soil, and feces. Nitrates in the environment can cause an excessive amount of growth algae and phytoplankton. Harmful algae blooms can secrete toxins, which can create unsafe drinking water and poison sea life. Previous studies have shown that specific nitrate reducing microorganisms can potentially reduce the levels of Nitrates. The objective of this study was to determine whether microorganisms can reduce nitrate levels in aquatic solutions. My hypothesis is if *Pseudomonas* are used to treat nitrate levels then nitrate levels in a test tube will decrease. This was researched by taking four test tubes, containing 1mL of nitrate solution. Two of the test tubes contained 1mL of *Pseudomonas* incubated in nutrient broth. Initially all the trials started out as 250 ppm nitrate levels. The control group #1 of no bacteria remained constant measuring to 250 ppm. While the control group of no bacteria #2, bacteria tubes 3 and 4 spiked after test 1 to 500 ppm and remained consistent. After taking data five times, I tightened the test tubes and left them in the incubator for a week to see if putting them in an anaerobic state would change the results. After a week the control group of no bacteria (#1) measured to 250 ppm Nitrate level, the control group of no bacteria (#2) measured to 500 ppm Nitrate level. Bacteria tubes 3 and 4 both measured to 500 ppm Nitrate level.

### Introduction:

Wastewater comes from the day to day life of bathing, flushing a toilet, laundry and washing dishes. But where does all that water go when it goes down the sink or drain? The wastewater can either go into a septic tank or a lagoon system. (University of Nebraska-Lincoln, 2019) But some residents have a sewer which then connects to a community sewer. Then it will end up at a treatment center to be cleaned, which then can be released into local waterways. After being released it can be used for many purposes such as agriculture and drinking water. (USEPA, 2002) Almost all water is treated before being reused. Not all wastewater comes from a house; it can also be storm runoff. Although many think that rain water is clean it is not. After flowing down streets, it can lift up harmful substances which can be dangerous for local water systems. Wastewater is very important to treat because it could be very dangerous for the health of humans, animals and waterways. (WSS, 2018) One thing that makes wastewater dangerous are nitrates.

Nitrates can occur in food which contains a healthy level but if the level gets too high then health concerns may arise. Nitrates are found in fertilizer, explosives, soil, many foods and lots of other things. Nitrates can get into water from many different things. Mostly nitrates come from runoff, fertilized soil, landfills and septic tanks. Nitrates in water have become a serious environmental problem. And if not treated properly can cause harm to many things. An abnormal amount of nitrates in wastewater can contaminate groundwater and cause the growth of algae. (Millipore, 2021 )

In aquatic environments, nitrates can stimulate the growth of algae. Algae blooms can also sometimes secrete which can cause unsafe drinking water, poison sea life and other

mammals, and cause the creation of dead zones. A dead zone is an area where no life can be of habitat and sustain remaining alive. (Aquamonitrix, 2021)

Bacteria can be either anaerobic or aerobic. Bacteria that are aerobic are oxygen consuming. But anaerobic bacteria are potentially the suit for reducing nitrates. The solution using anaerobic bacteria isn't the fastest way to reduce nitrates because the bacteria has to establish a population. (Gay, 2019) Nitrates are electron acceptors, the growth rate of denitrifiers depends on the concentration of nitrates in the environment. (Shukla et al., 2021)

#### Hypothesis:

If microorganisms, Pseudomonas, are used in wastewater then nitrate levels will be reduced.

#### Methods:

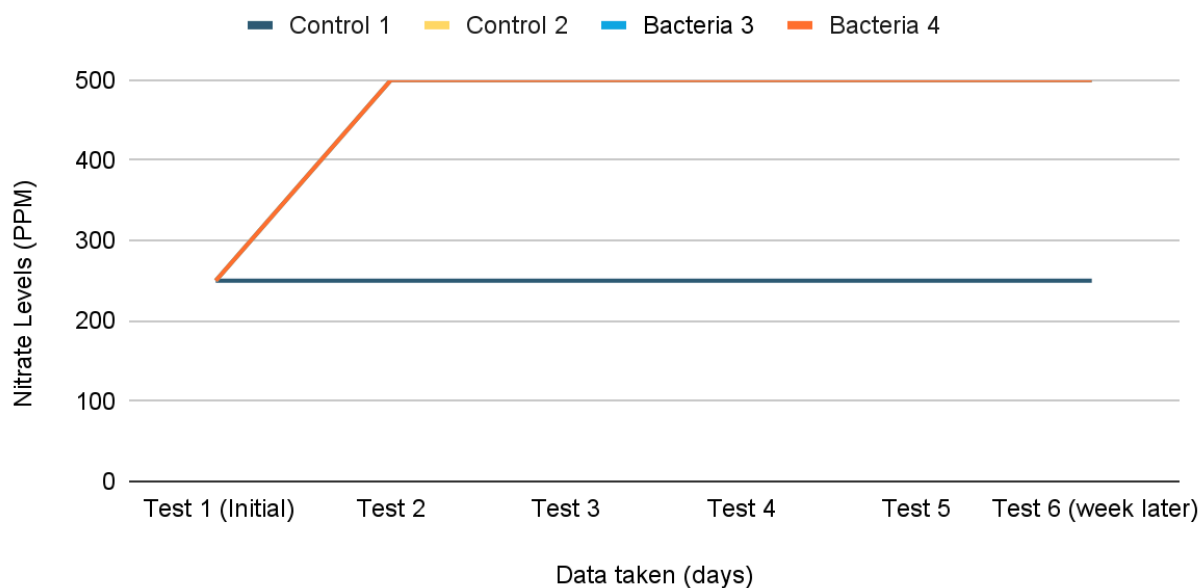
First, I created my culture of Pseudomonas. I used a sterile inoculating loop to gather Pseudomonas which were then placed in 10mL of nutrient broth. I repeated this four times. Then I agitated each test tube three times. Next I opened the lids of the test tubes a tiny bit. After that they were placed in the incubator to grow at 60 degrees fahrenheit. After four days of letting the microorganisms grow, I added 1 mL nitrate solution to four test tubes that contained broth solution. Two test tubes contained 1 mL of microorganisms solution. All test tubes were agitated three times, and tested for nitrates.

#### Results:

Initially all the groups started out the same with 250 ppm Nitrate level. This is because it was the first test where the nitrate solution was freshly put. The microorganisms did not have time to act upon. The control group of bacteria remained constant throughout the entire trial

measuring out to 250 ppm Nitrate level. While the second control group of no bacteria spiked to 500 ppm Nitrate level after Test 1. Along with the second control group of no bacteria, bacteria tubes 3 and 4 both spiked to 500 ppm Nitrate level after Test 1. These results remained consistent throughout the five tests. After taking data five times, I decided to tighten all the test tubes and leave them in the incubator for a week to see if putting them in an anaerobic state would change the results. After a week the control group of no bacteria measured to 250 ppm Nitrate level, the second control group measured to 500 ppm Nitrate level. Bacteria tubes 3 and 4 both measured to 500 ppm Nitrate level.

### The Effect of Microorganisms on Removing Nitrates in Wastewater.



Above, the graph represents each group and the measure of nitrate level for each test. Although there are four lines only two show because three groups measured the same through the experiment. The nitrate levels were measured in PPM. Data was taken a total of six times.

Test:	Control Group 1 of no Bacteria	Control Group 2 of no bacteria	Bacteria Tube 3	Bacteria Tube 4
1 (Initial)	250 ppm	250 ppm	250 ppm	250 ppm
2	250 ppm	500 ppm	500 ppm	500 ppm
3	250 ppm	500 ppm	500 ppm	500 ppm
4	250 ppm	500 ppm	500 ppm	500 ppm
5	250 ppm	500 ppm	500 ppm	500 ppm
6 (week later than 5)	250 ppm	500 ppm	500 ppm	500 ppm

The table above shares each data I received from each test.

#### Discussion:

The purpose of this experiment was to determine if microorganisms, specifically *Pseudomonas*, lower Nitrate levels in aquatic solutions. Originally I thought that if *Pseudomonas* was used to treat Nitrate levels in aquatic solutions, then the Nitrate levels would decrease. The data I received rejects my hypothesis. The control group of no *Pseudomonas* stayed at 250 ppm Nitrate levels throughout the entirety of the experiment. The control group 2 of no *Pseudomonas* increased to 500 ppm Nitrate level and remained consistent throughout the experiment. Bacteria tubes 3 and 4 both contained *Pseudomonas* and increased to 500 ppm Nitrate level. Originally I thought that the lack of variation was due to my test tubes being in an aerobic state when typically in a real environment it would be an anaerobic state. I continue to believe that is partially why variation between groups was slim but I also believe that if I expanded the trial longer maybe results would show a change. Running the trial longer not only would give the bacteria a change to populate but it would also increase validity.

### Limitations:

Some limitations I faced were the fact that Nitrates in aquatic environments are used to a more anaerobic environment. While in my experiment my solutions were in an aerobic environment, this could have altered the results I received.

### Future Studies:

In future studies I would like to look at a more ecological friendly and natural nitrate reducer with a larger sample size and a longer run trial. Along with a different way to test nitrates. I could also test drinking water and local waterways nitrate levels. Another idea could be to determine the effects of a nitrate water solution on the plant growth. Using nitrate water solution to water a plant and keeping track of growth in a plant.

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