LOGBOOK

2021 Science Fair

Bioremediation of Controlled Oil Spill with Microbes

In my project I will test if changing amounts of motor oil and nutrients in a controlled fresh-water oil spill with added microbes will affect the amount and speed the oil will be bioremediated.

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Schedule

Finalize Test Procedure: January 25th, 2021

Start Test: January 27th, 2021.

Finish Test: February 4th, 2021

Finalize Procedure for Phase 1: February 5th, 2021

Start Phase 1 Experiment: February 6th, 2021.

Finish Phase 1 Experiment: February 20th, 2021.

Finalize Procedure for Phase 2: February 26th, 2021.

Start Phase 2 Experiment: February 27th, 2021.

Finish Phase 2 Experiment: March 6th, 2021.

Daily Notes and Ideas

2020-12-14: Discussed the possibility of science fair with my science teacher, Mr. Krawchuk.

2020-12-23: backhoe fell through the ice at my grandparents' pond. During removal of the backhoe the diesel tank cap broke, and diesel spilled into the pond and onto the ice.

2020-12-24: Started researching how to remove the diesel from the pond – discovered oil eating microorganisms. This was interesting to me and thought of this as a possibility for Science Fair.

2021-01-05: Mr. Krawchuk (my science teacher) announced the science fair.

2021-01-10: Did more research on oil eating microorganisms and where to buy them – ordered oil-eating microbes (powder)

2021-01-11: Received an email saying that I made it into the CYSF!

2021-01-14: Oil-eating microbes arrived in the mail! Had first CYSF meeting with Mr. Krawchuk and other classmates – discussed project ideas, logbooks, scientific method, project expectations, due dates, forms, project divisions and topics etc.

2021-01-19: Emailed safety and ethics lead (Leslie Sears) and asked if my project was safe and allowed to complete, and approval was given to use the microbes.

2021-01-20: Did some background research and discovered the definitions of biodegradation, degraded, metabolic, bio stimulation, aerobic bacteria, aerobic biodegradation, anaerobic biodegradation, bioremediation, in-situ bioremediation, ex-situ bioremediation, bioreactors, apparatus and anatomical.

2021-01-21: Attended science fair club and continued to discuss experiments and learned about the online platform to place important information.

2021-01-24: Read articles and other papers on other experiments related to microbes and oil spills, decided what variables to change and what I am placing in each test tube, started writing a procedure, turned on the magnetic stirrer to make sure it works, did some research on what crude oil and petroleum are.

2021-01-25: Finalized test procedure and started basic materials list.

2021-01-27: Starting to revive a test batch of microorganisms for a test to see if amounts of nutrients and motor oil were appropriate.

2021-01-28: Divided culture into 10 different test tubes some with fertilizer, distilled water and motor oil

2021-02-04: One week after experiment test tubes with distilled water and fertilizer and 1mL of motor oil appeared to have the most bioremediation although it was difficult to measure because 1mL and 2.5mL spread onto of water in a test tube is hard to visually see a difference. The test tubes with no distilled Appeared to not have much bioremediation occurring. All the microbes went to the bottom of the test tubes. When the oil was bioremediated the oil turned a cloudy white colour. Throughout the entire experiment the test tubes were places on a heating mat.

2021-02-06 – Started reactivating Microbes

2021-02-07 Started Experiment and did starting measurements and observations.

2021-02-08 Did measurements of experiment, observations and swirled the test tubes around for 15seconds to represent water current.

2021-02-09 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-10 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-11 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-12 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-13 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-14 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-15 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-16 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-17 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-18 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-19 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-20 Did measurements of experiment, observations and swirled the test tubes around for 15seconds.

2021-02-21 Started analyzing data and creating tables and charts and collected water samples from my grandparents' pond for phase 2.

2021-02-22 Continued Analyzing data and creating tables and charts.

2021-02-23 did research on possible chemical reaction at the bottom of test tubes from phase 1

2021-02-24 Continued research and discovered that it is probably a reaction between the water and the motor oil allowing oxidization to occur or it is lipids.

2021-02-25 Started planning out phase 2 and created a draft procedure for phase 2 .

2021-02-26 – Created a hypothesis for phase 2 and edited procedure.

2021-02-27 Started reactivating microbes for phase two of experiment. Did 1g of microbes to 500mL of settled water.

2021-02-28 – started phase two, did measurements, observations, and swirls.

2021-03-01 - Did measurements, observations, and swirls of test tubes.

2021-03-02 - Did measurements, observations, and swirls of test tubes.

2021-03-03 - Did measurements, observations, and swirls of test tubes.

2021-03-04 - Did measurements, observations, and swirls of test tubes.

2021-03-05 - Did measurements, observations, and swirls of test tubes.

2021-03-06 - Did measurements, observations, and swirls of test tubes.

2021-03-07 – Started putting data into charts and graphs.

2021-03-08 – Continued putting data into chats and graphs.

2021-03-09 – Did a draft analysis.

2021-03-10 – Started thinking about video

2021-03-11 – Started PPT for my presentation.

2021-03-12 – Tried to start putting tables and graphs and other information into portal and realized that I cannot put in graphs unless they are in picture form and some tables do not fit on the screen. I also discovered that when I copy and paste my test the blue color that I have been using for headings does not show up and when I try to change the color all of the text goes to that color, and if I try to change the size of the text all of the text changes not the high lighted text. Filled out declarations.

2021-03-13 – Made hypothesis more concise for the portal.

2021-03-14 – Edited Work

2021-03-15 – Edited Work and printed out information for trifold

2021-03-16 Glued work on trifold and recorded my project video

2021-03-17 Reviewed my project with teacher and made last edits and submitted everything

Background Research – See also scanned notes from written notes

"What is Biodegradation?" (The Biology Notes)

- "Microorganisms play a key role in the decomposition of organic materials accumulated in the environment."
- "They are the recyclers of nutrients in the soil."
- "Almost all biogeochemical cycles are driven by the indigenous microbial population in the soil."
- "Biodegradation is the process in which organic compounds are degraded or broken down by the microorganisms."
- "It is an important process that replenishes the environment with nutrients."
- "Microorganisms degrade organic material for their growth and metabolism."
- "As a result, complex organic substances are converted into carbon dioxide and water."

Aerobic biodegradation is done by aerobic microorganisms when an adequate supply of oxygen is available for their activity.

- It is a rapid method that degrades the contaminants completely when compared to anaerobic biodegradation.

Anaerobic biodegradation takes place in the absence of oxygen. Its pathway has four major steps:

Hydrolysis
Acidogenesis
Acetogenesis
Methanogenesis

Organic substances are subjected to anaerobic digestion and converted into carbon dioxide and methane.

"Oleophilic microbes (Oil Eating Microbes) are normally found in marine environments." (Edvotek)
OEMs eat hydrocarbons (compounds that make up crude oil) Microbes separate hydrocarbons into fatter acids which are split into carbon atoms which have been reused by the microbes in metabolism. Oil is broken metabolites, carbon dioxide and water.

- The final by-products of bioremediated oil are carbon dioxide, water, trace carbon, and lipids (which are white fatty acids). The lipids are a source of food for larger organisms to survive. (UltraTech)
- Definition of Oxidization: "combine or become combined chemically with oxygen" (Oxford Languages)

Oil Spills

It is important that when oil spills happen that they are cleaned up in the most effective way that is done with consideration to the environment. According to the EPA "Oil spills occurring in freshwater bodies are less publicized than spills into the ocean even though freshwater oil spills are more frequent and often more destructive to the environment." It is important to properly clean the site of oil as we source our drinking water from many freshwater bodies of water, the water bodies and their surroundings are used as nesting grounds and food sources for many biotic freshwater organisms. All freshwater organisms and vegetation are vulnerable to the deadly and toxic effects oil spills can have on them and their environment. According to the EPA "Standing water such as marshes or swamps with little water movement are likely to incur more severe impacts than flowing water because spilled oil tends to "pool" in the water and can remain there for long periods of time." It is even more important to properly clean the areas because it could save lives for all of the creatures living in the area.

"Spilled oil can harm living things because its chemical constituents are poisonous. This can affect organisms both from internal exposure to oil through ingestion or inhalation and from external exposure through skin and eye irritation. Oil can also smother some small species of fish or invertebrates and coat feathers and fur, reducing birds' and mammals' ability to maintain their body temperatures." (Office of Response and Restoration)

Hydrocarbons

Crude oil is unrefined petroleum, "Crude oil, also known as petroleum, is an energy-rich liquid consisting mainly of hydrocarbons." (Government of Canada)

"Petroleum, also called crude oil, is a fossil fuel. Like coal and natural gas, petroleum was formed from the remains of ancient marine organisms, such as plants, algae, and bacteria. Over millions of years of intense heat and pressure, these organic remains (fossils) transformed into carbon-rich substances we rely on as raw materials for fuel and a wide variety of products." (National Geographic)

The Process of Oil Breakdown

Biodegradation is a natural process that occurs in the environment.

"Bioremediation is an engineered technique applied by humans to clean the environment." (The Biology Notes)

Aerobic biodegradation is done by aerobic microorganisms when an adequate supply of oxygen is available for their activity.

- It is a rapid method that degrades the contaminants completely when compared to anaerobic biodegradation.

Anaerobic biodegradation takes place in the absence of oxygen. Its pathway has four major steps:

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- The final by-products of bioremediated oil are carbon dioxide, water, trace carbon, and lipids (which are white fatty acids). The lipids are a source of food for larger organisms to survive. (UltraTech)

Oil Eating Microbes

"Oleophilic microbes (Oil Eating Microbes -OEM's) are normally found in marine environments." (Edvotek)

OEMs eat hydrocarbons (compounds that make up crude oil) Microbes separate hydrocarbons into fatter acids which are split into carbon atoms that have been reused by the microbes in metabolism. Oil is broken metabolites, carbon dioxide, and water.

The 6 conditions that affect microbe growth (Quizlet)

- Reservoir. Environment where most microbes grow.
- Food. Water and nourishment.
- Oxygen. Most need oxygen to survive.
- Darkness. Warm and dark environments is needed.
- Temperature. Most grow best at body temperature.
- Moisture. Grow well in moist places.

"The addition of fertilizer during the bioremediation has been proved very effective and essential to stimulate microbial growth as well as contaminates removal. However, inhibitory effects have also been observed when the concentration of nutrients was excessive, mostly due to their toxicity to microbial species."

Definitions

- Oxidization: "combine or become combined chemically with oxygen" (Oxford Languages)
- Biodegradation: (The Biology Notes)
    - "Microorganisms play a key role in the decomposition of organic materials accumulated in the environment."
    - "They are the recyclers of nutrients in the soil."

  - "Almost all biogeochemical cycles are driven by the indigenous microbial population in the soil."
  - "Biodegradation is the process in which organic compounds are degraded or broken down by the microorganisms."
  - "It is an important process that replenishes the environment with nutrients."
  - "Microorganisms degrade organic material for their growth and metabolism."
  - "As a result, complex organic substances are converted into carbon dioxide and water."
- Microbes: "tiny living things that are found all around us and most are too small to be seen by the naked eye. They live in water, soil, and in the air." (NCBI)
- Lipids: Fatty Acids or by-products that are insoluble in water but soluble in organic solvents. (Oxford Languages) **"What is Biodegradation?" (The Biology Notes)**
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Hypothesis

First Edited Draft

Phase #1

I think that the test tubes containing 3g of fertilizer might impact the amount of bioremediation occurring because some fertilizer can stimulate microbial growth and contaminant removal, but too much nutrients can be toxic to microbes. I could find no research on how much fertilizer the microbes can withstand, but because this is my highest amount of fertilizer that I am using I will assume that there will be less bioremediation in the test tubes containing 3g of fertilizer. I think that the test tubes containing 1g of fertilizer will stimulate microbial growth and contaminant removal the most because I think that the 3g of fertilizer may be more toxic for the microbes and the 0.5g will not do much to stimulate microbial growth and contaminant removal.

I think that the test tubes containing 2.5mL of motor oil all the oil will be bioremediated by the microbes once the two weeks have past and possibly the microbes in these test tubes might die because they need food to survive. I think that the microbes might eat all the motor oil before the two-week experiment is complete. I think that the test tubes containing 5mL of motor oil I think almost all the motor oil will be eaten because although it is double the amount of motor oil as the lowest amount, I think that the microbes will be able to eat almost all of it and that they will not run out of food before the two-week experiment is complete. I think that the test tube containing 8mL of motor oil will have more oil that is not bioremediated because there is so much oil in these test tubes. I think that there will be just shy of double the amount of oil that is not white and cloudy as the amount of not bioremediated oil in the test tubes containing 5mL of motor oil.

Phase #2

I think that the test tubes with 3g of fertilizer and the test tubes with 0 fertilizer will have the most bioremediation occur. I think this based on my results of the last experiment because these test tubes did the best, I am now varying the amounts of fertilizer a bit to see if my theory is true, that either if you add a larger amount of fertilizer or no fertilizer, they will have similar results. I think that this is useful because when cleaning up an oil spill in a site you do not want to add nutrients (fertilizer) to the site because it can be harmful to the organisms and flora living in the area. I do not think that the test tubes with 1.5g of fertilizer will help to stimulate bioremediation as much as the others because in phase 1 test tubes with 1 & 2g of fertilizer they did slightly worse than the test tubes containing 3 or 0 g of fertilizer.

Second Edited Draft

Phase #1 – Group 1

- I think that the test tubes with 3g of fertilizer might have the most bioremediation because nutrients, if used in the right ratio are proven to help stimulate microbial growth and contaminant removal, but I am not sure if 3g nutrients to 25mL of microbes might possibly be toxic, and in this case, there might be not bioremediation.

- I think that the test tube with 1g of fertilizer will have a lot of bioremediation, but not as much as the test tubes with 3g of fertilizer if it is not toxic to the microbes. I think this because there is less fertilizer which might mean that there is less nutrients to stimulate bioremediation.

• I think that the test tubes containing 0.5g of fertilizer will not do much to stimulate microbial growth and contaminant removal. I think that these test tubes would have similar results to the controls with zero nutrients because there is very little nutrients.

Phase #1 – Group 2

• I think that the test tubes containing 2.5mL of motor oil will be completely bioremediated by the time the two-week period if up, I think this because these test tubes have the least amount of motor oil, and from my research when you remove some of the oil before getting the remaining oil bioremediated it is more successful.

• I think that the test tubes containing 5mL of motor oil will be almost completely bioremediated by the time the two-week period is up, I think that there might still be some non-bioremediated oil remaining because there is double the amount of motor oil as in the first test tubes and because with more motor oil the bioremediation process is less successful and takes more time.

• I think that the test tubes containing 8mL of motor oil will have over 30% if the oil be non-bioremediated.

Phase #2

•I think that the test tubes with 3g of fertilizer and the test tubes with 0 fertilizer will have the most bioremediation occur. I think this based on my results of the last experiment because these test tubes had the most bioremediation occur.

• I do not think that the test tubes with 1.5g of fertilizer will help to stimulate bioremediation as much as the test tubes containing none or 3g of fertilizer. In phase 1, test tubes with 1 & 0.5g of fertilizer had less bioremediated oil than the test tubes containing 3 or 0 g of fertilizer.

Final Hypothesis

Phase #1 – Group 1 – Varying Amounts of Nutrients

- **Lowest Nutrients (0.5g)**: I think that this amount of nutrients will not do much to stimulate microbial growth and contaminant removal and would have similar results to the controls with zero nutrients.
- **Medium Nutrients (1g)**: I think that this amount of nutrients will have the most bioremediation because I think this amount will not be toxic to the microbes but will still stimulate bioremediation and microbe reproduction.
- **Highest Nutrients (3g)**: From my research I know that high amounts of nutrients may kill the microbes but I am not sure what ratio will be toxic. This nutrient is the highest of my tests so I think it is most likely to have toxic effects.

Phase #1 – Group 2 – Varying Amounts of Motor Oil

- **Lowest Motor Oil (2.5mL):** I think this amount of motor oil will be completely bioremediated within the two-week period. From my research, bioremediation is most successful when as much source contaminant and free oil is removed.
- **Medium Motor Oil (5mL):** I think this amount of motor oil will only be partially bioremediated by the time the two-week period is up. The thicker oil column will mean the bioremediation will take longer because there is more oil to consume and because it is harder for the microbes to access the top of the oil layer.

- **Highest Motor Oil (8mL):** I think this amount of motor oil will also only be partially bioremediated and even more oil will be remaining than in the medium test.

Phase #2 – Pond Water – Varying Amounts of Nutrients

- **No Nutrients (0g)**: I think that no nutrients will still have a high level of bioremediation based on my phase 1 test.
- **Medium Nutrients (1.5g)**: I think that this amount of nutrients will not have the least amount of bioremediation based on results from my phase 1 test.
- **Highest Nutrients (3g)**: I think that this amount of nutrients will have the highest level of bioremediation. Phase 1 showed that this amount of nutrients is not toxic and stimulates microbial reproduction and bioremediation.

Materials List

Materials List

- 1,000 mL beaker
- Tin foil
- 2 g Oil Eating Microbe freeze dried powder (Edvotek)
- 1,000 mL of cold tap water – let sit for 3 hours to let the chlorine dissipate.
- 4cm plastic coated magnetic stir bar
- Inttlab magnetic stirrer
- Disinfectant wipes
- Scale that can measure less than 2g.
- Small container
- Plastic spoon
- Paper towel
- Nitrile gloves
- Apron
- Googles
- 30 test tubes
- 30 rubber size 5 lids for test tubes
- 2 test tube racks
- Seed-starting mat
- Un-Used Motor Oil
- Fertilizer (20-8-8)
- 2mL pipette
- 20mL pipette
- Glass Stir Sticks
- Distilled water

- Ruler
- Graduated Cylinders Different Sizes (2mL, 5mL, 10mL)

Experimental Procedure/Method

Edited Draft of Procedure for Phase 1

1. Calibrate scale.
2. Wash Hands
3. Wash equipment with soap and water and triple rinse
4. Put on nitrile gloves, goggles, apron, and tie hair back.
5. Add **2g** of Oil Eating Microbe (OEM) powder to 1,000mL of cold tap water into a 1,000mL beaker.
6. Place 4cm plastic-coated magnetic stir bar inside of the beaker.
7. Cover opening of the beaker with tin foil.
8. Place flask on a magnetic stirrer set at 200rpm (rotations per minute) and let stir for 24 hours or until cloudy **Source of error because my stirrer does not show the speed, so it is approximately 200rpm.**

After 24 hours:

9. Place 30 test tubes into stands
10. Label each test tube. 1A-1O and 2A-2O
11. Pour **30mL** of distilled water into 30 test tubes.
12. Dissolve **2g** of nutrients into 15 test tubes – fertilizer 20-8-8 (1A through 1O)
13. Pour **5mL** of motor oil into the other 15 test tubes. (2A though 20)
14. Pour **2.5 mL** of motor oil into test tubes 1A, 1G, 1H, and 1I.
15. Pour **5mL** of motor oil into test tubes 1B, 1J, 1K, and 1L.
16. Pour **8mL** of motor oil into test tubes 1C, 1M, 1N and 1O.
17. Dissolve **1g** nutrients (approximately 1/4 tsp) into test tubes 2A, 2G. 2H, and 2I.
18. Dissolve **2g** nutrients (approximately ½ tsp) into test tubes 2B, 2J, 2K and 2L.
19. Dissolve **3g** nutrients (approximately ¾ tsp) into test tubes 2C, 2M, 2N, 2O.
20. To test tubes 2D – 2O and 1D-1O add **25mL** of microbe mixture (do not stop the stirring process until ready to pour inside test tubes)

*Test tubes 1A-1C should only have 30mL distilled water, 2g nutrients, 25mL microbe mixture, and variations of motor oil (2.5 1A, 5 1B and 8 1C) and test tubes 2A, 2B, and 2C should only have 30mL distilled water, 5mL motor oil, 25mL microbe mixture, and variations of fertilizer (1 2A, 2 2B, and 3 2C)

23. Using an mm ruler measure the amount of motor oil per test tube and right down in chart, **does not include cloudy white bioremediated oil**
24. Place the test tubes on a seed-starting mat set at approximately 26 degrees C.
25. Each day for 14 days measure the amount of oil and any observations that you may have
26. Each day shake the test tubes for 15 seconds in all directions to represent water current
27. On the 14th day after finishing your observations soak the microbes in 10% bleach and 90% water for 30 minutes then dispose of in the garbage

28. Disinfect all surfaces and equipment.
29. Dispose of gloves after each use and thoroughly wash hands.

Final Procedures:

Phase 1

1. Calibrate scale.
2. Wash Hands
3. Wash equipment with soap and water and triple rinse
4. Put on nitrile gloves, goggles, apron, and tie hair back.
5. Add **2g** of Oil Eating Microbe (OEM) powder to 1,000mL of cold tap water into a 1,000mL beaker.
6. Place 4cm plastic-coated magnetic stir bar inside of the beaker.
7. Cover opening of the beaker with tin foil.
8. Place flask on a magnetic stirrer set at 200rpm (rotations per minute) and let stir for 24 hours or until cloudy **Source of error because my stirrer does not show the speed, so it is approximately 200rpm.**

After 24 hours:

10. Place 30 test tubes into stands
11. Label each test tube. 1A-1O and 2A-2O
12. Pour **30mL** of distilled water into 30 test tubes.
13. Dissolve **2g** of nutrients into 15 test tubes – fertilizer 20-8-8 (1A through 1O)
14. Pour **5mL** of motor oil into the other 15 test tubes. (2A through 20)
15. Pour **2.5 mL** of motor oil into test tubes 1A, 1G, 1H, and 1I.
16. Pour **5mL** of motor oil into test tubes 1B, 1J, 1K, and 1L.
17. Pour **8mL** of motor oil into test tubes 1C, 1M, 1N, and 1O.
18. Dissolve **1g** nutrients (approximately 1/4 tsp) into test tubes 2A, 2G. 2H, and 2I.
19. Dissolve **2g** nutrients (approximately ½ tsp) into test tubes 2B, 2J, 2K, and 2L.
20. Dissolve **3g** nutrients (approximately ¾ tsp) into test tubes 2C, 2M, 2N, 2O.
21. To test tubes 2D – 2O and 1D-1O add **25mL** of microbe mixture (do not stop the stirring process until ready to pour inside test tubes)

*Test tubes 1A-1C should only have 30mL distilled water, 2g nutrients, 25mL microbe mixture, and variations of motor oil (2.5 1A, 5 1B, and 8 1C), and test tubes 2A, 2B, and 2C should only have 30mL distilled water, 5mL motor oil, 25mL microbe mixture, and variations of fertilizer (1 2A, 2 2B, and 3 2C)

23. Using an mm ruler measure the amount of motor oil per test tube and right down in the chart, **does not include cloudy white bioremediated oil**
24. Place the test tubes on a seed-starting mat set at approximately 26 degrees C.
25. Each day for 14 days measure the amount of oil and any observations that you may have
26. Each day shake the test tubes for 15 seconds in all directions to represent the water current
27. On the 14th day after finishing your observations soak the microbes in 10% bleach and 90% water for 30 minutes then dispose of in the garbage
28. Disinfect all surfaces and equipment.
29. Dispose of gloves after each use and thoroughly wash hands.

Phase 2

1. Calibrate scale.
2. Wash Hands
3. Wash equipment with soap and water and triple rinse
4. Put on nitrile gloves, goggles, apron, and tie hair back.
5. Add 1g of Oil Eating Microbe (OEM) powder to 500mL of cold tap water (that has been set out to sit for a couple of hours) into a 1,000mL beaker.
6. Place 4cm plastic-coated magnetic stir bar inside of the beaker.
7. Cover opening of the beaker with tin foil.
8. Place the beaker on a magnetic stirrer set at 200rpm (rotations per minute) and let stir for 24 hours or until cloudy ***Source of error because my stirrer does not show the speed, so it is approximately 200rpm.**

After 24 hours:

9. Place 13 test tubes into stands
10. Label each test tube. 3A to 3M
11. Pour 30mL of pond water into 13 test tubes.
12. Dissolve 1.5g of nutrients (fertilizer 20-8-8) into test tubes 3H, 3I & 3J
13. Dissolve 3g nutrients (approximately ¾ tsp) into test tubes 3K, 3l & 3M.
14. Add 25mL of microbe mixture to all test tubes except 3A & 3B. (do not stop the stirring process until ready to pour inside test tubes)
15. Using a pipette measure out and add 2.5mL of motor oil to all test tubes except 3C & 3D

Here is what the test tubes should have in them up to this point:

All

- 30mL pond water (except control #1)
- 25ml microbes (except control #1)
- 2.5mL motor oil (except control #2)

Control #1 x2 (3A and 3B)

- No Microbes
- 55mL pond water
- 2.5mL oil

Control #2 x2 (3C and 3D)

- No Oil
- 25mL microbes
- 30mL pond water

Test #1 (3E, 3F & 3G)

- 0g nutrients

Test #2 (3H, 3I, & 3J)

- 1.5g nutrients

Test #3 (3K, 3L, & 3M)

- 3g nutrients

16. Using an mm ruler measure the amount of motor oil per test tube and right down in the chart, does not include cloudy white bioremediated oil.
17. Place the test tubes on a seed-starting mat set at approximately 26 degrees C.
18. Each day for 7 days measure the amount of remaining motor oil and any observations that you may have
19. Each day swirl the test tubes for 15 seconds in all directions to represent the water current
20. On the 14th day after finishing your observations soak the microbes in 10% bleach and 90% water for 30 minutes then dispose of in the garbage
21. Disinfect all surfaces and equipment.
22. Dispose of gloves after each use and thoroughly wash hands.

Data Collection Phase 1

The following measurements were collected during the different phases of the experiment. The data was entered into excel.

Raw Data from Phase #1: Group 1 (Varying Oil Amount)

		Motor Oil amount in mm													
Test Tube:		Day #1	Day #2	Day #3	Day #4	Day #5	Day #6	Day #7	Day #8	Day #9	Day #10	Day #11	Day #12	Day #13	Day #14
Control 1 No Microbes	1A	6	6	6	6	6	6	6	6	6	6	6	6	6	5
	1B	10	10	9	8	8	8	8	8	8	8	8	8	8	8
	1C	14	14	14	13	13	13	13	13	13	13	12	12	12	12
Control 2 No Oil	1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1E	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1F	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.5mL Oil	1G	6	5	4	4	3	3	3	3	3	2	2	2	2	2
	1H	6	5	4	4	3	3	3	3	3	2	2	2	2	2
	1I	6	5	4	4	3	3	3	3	2.5	2	2	2	2	2
5mL Oil	1J	10	9	8	8	7	7	7	7	8.5	6	5	4	4	3
	1K	10	9	8	8	7	7	7	7	8.5	6	5	4	3	3
	1L	10	9	8	8	7	7	7	7	8.5	6	5	4	3	3
8mL Oil	1M	14	13	12	12	11	11	11	11	10	10	10	9	9	9
	1N	14	13	12	12	11	11	11	11	10	10	9.5	9	9	9
	1O	14	13	12	12	11	11	11	11	10	10	10	9	9	9

All test tubes for Group 1 contain:
- 30mL distilled water (except Control 1 with no microbes, they have 55mL distilled water)
- 25mL microbes (except Control 1)
- 2g nutrients

Raw Data from Phase #1: Group 2 (Varying Nutrient Amount)

		Motor Oil amount in mm													
Test Tube:		Day #1	Day #2	Day #3	Day #4	Day #5	Day #6	Day #7	Day #8	Day #9	Day #10	Day #11	Day #12	Day #13	Day #14
Control 1 No Microbes	2A	8	8	8	8	8	7	6	6	6	5	3	3	5	5
	2B	8	8	8	8	8	7	6	6	6.5	5	4	4	4	5
	2C	8	8	8	8	8	7	6	6	6.5	5	5	5	5	5
Control 2 No Nutrients	2D	8	7	6	6	6	6	6	6	5	4	3	3	2	2
	2E	8	7	6.5	6	6	6	6	6	5	4	3	3	2	2
	2F	8	7	6	6	6	6	6	6	5	4	3	3	2	2
1g Nutrients	2G	8	7	7	7	6	6	6	6	7	6	3	3	3	3
	2H	8	7	7	7	6	6	6	6	7	6	3	3	3	3
	2I	8	7	7	7	6	6	6	6	7	6	3	3	3	3
2g Nutrients	2J	8	7	7	7	6	6	6	6	6	5	4	3	3	3
	2K	8	7	7	7	6	6	6	6	6	5	4	3	3	3
	2L	8	7	7	7	6	6	6	6	6	5	4	3	3	3
3g Nutrients	2M	8	7	7	7	6	6	6	6	6	4	3	3	2	2
	2N	8	7	7	7	6	6	6	6	6	4	3	2	2	2
	2O	8	7	7	7	6	6	6	6	5	4	3	2	2	2

All test tubes for Group 2 contain:
- 30mL distilled water (except Control 1 with no microbes, they have 55mL distilled water)
- 25mL microbes (except Control 1)
- 5mL oil

Data Collection Phase 2

Raw Data from Phase 2 (Pond Water)								
Motor Oil amount in mm								
Test Tube:		Day #1	Day #2	Day #3	Day #4	Day #5	Day #6	Day #7
Control 1 No Microbes No Nutrients	3A	5	5	5	5	5	5	5
	3B	5	5	5	5	5	5	5
Control 2 No Oil No Nutrients	3C	0	0	0	0	0	0	0
	3D	0	0	0	0	0	0	0
0g Nutrients	3E	5	3	2	2	2	1	0.5
	3F	5	3	2	2	2	1	0.5
	3G	5	3	2	2	1.75	1	0.5
1.5g Nutrients	3H	5	4	4	3	2.5	2	1
	3I	5	4	3	3	2.5	2	1
	3J	5	4	3	3	2.5	2	1
3g Nutrients	3K	5	4	3	2	1	0.5	0
	3L	5	4	3	2	1	0.5	0
	3M	5	3.5	3	2	1	0.5	0
All test tubes for Phase 2 contain:								
- 30mL pond water (except Control 1 with no microbes, they have 55mL pond water)								
- 25mL microbes (except Control 1)								
- 2.5mL oil (except Control 2)								

Raw Observations of Phase 1

2021-02-07 – 2021-02-20

Day #1

The oil is all a honey color and is sitting on top of the water/microbes/fertilizer. The microbes have all sunk to the bottom of the test tubes. No bioremediation has occurred so far and the amounts in each test tube vary by less than 2mL because it was difficult getting every drop of oil out of the vials and into the test tubes and some of the microbes got stuck on the vials as well when pouring into the test tubes. The test tubes all have lids on them which are not pushed in to allow oxygen to get in. I have also noticed that when I swirl the test tubes for 15 seconds the motor oil, bioremediated oil, fertilizer and microbes all mix together.

Day #2

Today the microbes appear to have "crawled" up the test tube to the motor oil. It appears that the microbes have degraded some of the oil as all of the measurements done with the ruler have dropped 1mm. Also, in most of the test tubes there is a small amount of bioremediated oil that has turned a cloudy white colour. In some of the test tubes this cloudy bioremediated oil is floating inside of the motor oil, others it is on top and some of them it appears to have sunk down and is floating in water and microbe mixture.

Day #3

It appears that the nutrients do affect bioremediation. 2D, 2E, and 2F contain zero nutrients and bioremediation continued to occur and the amount of motor oil continued to drop by a mm. The test tubes containing 1g, 2g and 3g of fertilizer appeared to have slowed down the speed of bioremediation as there was no drop in amount of motor oil. All the "1" tests continued to drop 1mm except for the controls and the ones that do not contain any motor oil. This could mean that even though there are varying amounts of motor oil in the different test tubes does not affect the speed of bioremediation. The 2g of fertilizer appear to either increase amounts of bioremediation or do nothing, I cannot tell yet as all the test tubes contain the same amount of nutrients.

Day #4

Today there does not appear to be a huge difference between Day #3 and Day #4. I did notice that there is a lot more bioremediated oil than there was yesterday. I think that in my measurements it included some of the bioremediated oil. It looks like in the test tubes there is a lot of oil that is a cloudy white color that looks like it has dripped down the layer of motor oil. I am not sure how to describe it but the bioremediated oil is mixed into the motor oil, so i think that the measurements that I took today probably included some of the bioremediated oil since it is "floating" in the layer of motor oil. There are a lot of microbes that appear to be white sitting at the bottom of the test tubes. I am not sure if it is just some microbes or if that is some of the bioremediated oil that has sunk to the bottom of the test tube.

Day #5

Today in some of the test tubes it appears that some of the microbes are "floating" in some of the distilled water closer to the bottom of the test tubes. Some microbes are on above of the motor oil as if the motor oil has been bioremediated and dropped in size, and some of the microbes stayed above the layer of motor oil as if they were "left behind" after the oil was bioremediated. In some of the test tubes with less motor oil I can notice a significant color difference between the test tubes with more motor oil. It seems like the oil has turned a lighter color or that with less oil the color is lighter than when there is more oil. I can notice a significant difference between today and yesterday with the amount of oil and the color of the motor oil. It seems like the microbes took a little 'break" with bioremediation rates because the amount of oil that was being bioremediated seemed to plateau. Today there seemed to be a drop of amounts of motor oil, I am not sure why, but it might be that the microbes had to "digest" their food before eating more.

Day #6

Now the colour of the oil appears to have "dulled" it is not as shiny and glossy as it used to be, and the color seems to be more diluted and grayer. I think that this is happening because although there is no drop in amounts of motor oil today, I cannot just measure the motor oil as it appears that the bioremediated oil seems to be mixed in with the motor oil in random "globs." In test tube 1F there appears to be a spot of the white/grey mold floating on the surface. I am not sure where the mold came from or if it is even mold, but it surprised me. I have also noticed condesation in some of the test tubes in some the test tubes are almost completely clear except for a couple of microbes stuck to the sides, but in some test tubes there are large water droplets clinging to the sides of the test tubes. I notice it the most in test tubes 1I, 1H & 1G. These are the test tubes with the least amount of oil and the colour appears to be the lightest it is almost described as cloudy butter, but in liquid form. I have also noticed that in test tubes 1C, 1O, 1N, 1M, and 2C the colour appears to look like light/golden corn syrup colour as water. I think that all "1's" has that colour because they have the largest amount of motor oil, but I am not sure why 2C has a similar colour to the motor oil when it has the same amount of motor oil as the rest of the "2's" and they do not have that golden/light syrup colour. I see no difference in the amount of motor oil, although I am a bit worried that because the microbes are in test tubes, they are not getting their required amount of oxygen to survive. I am worried that if there is not enough oxygen the microbes will not be able to continue bioremediating the motor oil.

Day #7

Today the motor oil seems to be very similar to yesterday. Almost all the measurements were the same. I am beginning to get worried that the microbes are not getting enough oxygen and are dying. I am also thinking that that bioremediated oil may still be floating in the oil, but I cannot see very much of the cloudy white bioremediated oil. I think that this might be because of the liquid is cloudy, but I think that the motor oil is not getting bioremediated as much as it was originally. At the bottom of the test tubes almost all the microbes sitting at the bottom of the test tubes are turning white, I am not sure what it indicates, but I am still worried that the microbes are dying. I am worried that if I take off the lids of the test tubes it will change the variable since I thought that the microbes would be getting enough oxygen because I did not push on the lids of the test tubes because they have gotten stuck in the test tubes before. I have also noticed that the possible "mold spot" is still there and may be getting larger. I do not know how the mold got there, but I hope that the mold does not affect the bioremediation rates.

Day #8

When I looked at the test tubes from a different angle below 180 degrees/straight on I noticed that there has been a lot of bioremediation it appears that in majority of the "2" test tubes around 2/3 of the oil has be bioremediated and turned a cloudy white colour. It is very interesting that the bioremediated oil is almost in a dome, so when you do not look at it at the appropriate angle it just looks like the glossy motor oil has dulled and the colour has lightened, but it has just been bioremediated. So, all the measurements have contained some bioremediated oil. Tomorrow my measurements will try to only measure the remaining motor oil which means that most likely there will be a large drop in amounts.

Day #9

Some test tubes the motor oil and the bioremediated oil seems to be uneven around at some points in the test tube. In most test tubes you can see the microbes at the bottom of the layer of bioremediated oil, and it appears that they are eating/breaking down more of the motor oil. There is condesation on most of the test tubes, but 1I, 1H and 1G have the most. In some of the test tubes (specifically 1O, 1N and 1M) the motor oil is still glossy and almost the colour of canola oil. In some of the test tubes in the oil there is bits of bioremediated oil in the middle in the non-bioremediated oil mainly in test tubes 10,1N, and 1M. In test tubes 2A, 2B, and 2C I notice that there is some bubbling of the bioremediated oil. I also noticed that while measuring that some of the measurements are different depending on what angle and how much light you have while doing the measurements. This is not at variable that I considered so the measurements may be slightly off. I have also noticed that in some test tubes (specifically 1I, 1H and 1G) all of the motor oil ahs turned a cloudy light-yellow colour and I think that possibly all of the oil has been bioremediated as these test tubes had the least amount of motor oil to start and the colour is the lightest. I have also noticed that 2F, 2E, and 2D at the bottom of the test tubes the microbes are still brown, but the rest of the test tubes seem to have almost a white coating over them.

Day #10

I noticed that when I mixed/swirled the test tubes today the bioremediated oil and the motor oil almost combined so that you cannot tell a difference between the motor oil and the bioremediated oil. I think that possibly the white mixture covering some of the microbes at the bottom of the test tubes might be fertilizer or it might be a by-product from the bioremediation. It is confusing me why the test tubes 1O, 1N and 1M have had similar amounts of bioremediation yet the motor oil seems just as shiny as when I first poured it into the test tubes. Also, there is not much bioremediated oil, just a small thin layer that is very uneven and almost seems to be in globs. I also noticed that in test tube 1E there seems to be a layer of white at the top of the liquid. I am not sure what it is but I did notice that it went away when I swirled the test tubes today.

Day #11

I am not sure why the test tubes 1O, 1N and 1M have not have very much bioremediation, when I look at the test tubes the bioremediated oil seems to be a thin layer and it seems to be clinging to the sides of the test tubes. I also noticed that test tubes 1A, 1B, and 1C seem to have that white substance at the bottom of the test tube just like the rest of the test tubes even though these test tubes do not contain any microbes. I have also noticed that the liquid below the layer of motor oil in some of the test tubes seem to have a yellow hue compared to the other test tubes where the liquid it clear. I have also noticed that the condesation on the sides of the test tubes seems to have dried out in some areas and has turned white. I am not sure why it turned white, but I think it might have dried out because the air/water might have evaporated from the heating mat the test tubes have been sitting on for the last 11 days

Day #12

Today the color of the motor oil seems to be mostly a pale, faded light yellow colour and I think that in most of the test tubes almost all of the motor oil has been bioremediated. I am still very confused on why the test tubes 1O, 1N and 1M have not had very much bioremediation and the bioremediated oil seems to be bubbling and almost clinging to the sides of the test tubes. I have an idea of what the white liquid at the

bottom of the test tube might be, I think that it might be the result of a chemical reaction creating the white goop. I have also noticed that now in test tubes 2G-2O and 1E-1O the liquid (mostly distilled water) seems to have turned a light pale cloudy yellow/green colour. I am not sure why it has turned this colour or why not all of the test tubes have this colour, but I thought that it is really interesting that only some of the test tubes have this colouring of the water. There is also more mold in test tubes 1F, 1E and 1D. Test tube 1D has small circular spots of mold that are grey on the outside and brown/black on the inside. It also appears that they are above the water. In test tube 1E the mold is all white and in a ring around the edges of the test tube. It is not circular and seems to be many small dots all growing around the test tube above the water in a thin line. In test tube 1F there is a ring of mold almost identical to test tube 1E, but there is also some larger clumps of orange/yellow mold that is inside of the water. Also, the "ring" of mold is thicker in test tube 1F than in test tube 1E. I have also noticed that the water in test tube 1E appears to be almost a brown ombre. It starts of as a light yellow and then goes down to a dark cloudy brown. I am not sure why this water colour is different from the rest or what could have caused it.

Day #13

I have noticed that in the test tubes containing the least amount and moderate amount of oil have had the most success with the bioremediation. In the test tubes with 8mL of motor oil even though there was still a decrease when a compared these test tubes to the other test tubes there was a difference in the colour of the mixture and most likely is a chemical reaction and not bioremediation. I think that if this were a real-world situation, I think from my observations it would work best if you used another option to remove most of the oil either my burning it off, by an oil boom or with other methods and then adding the microbes to the site when there is only a skim of water left on the surface for the microbes to break down. I think a similar thing has occurred in test tubes 1B and 1C as these measurements continue to drop even though there is no microbes and I think that it might be a chemical reaction causing the white liquid that seems similar to bioremediated oil. I have also noticed when comparing test tubes 1D, 1E. and 1F I think that the microbes in test tube 1D are most likely dead as the liquid is clear compared to 1F and 1E which the liquid has either a brown (1E) or green/yellow hue (1F)

Day #14

Today the motor oil in test tube 1A seems to be a light cloudy yellow colour. This test tube seems to have almost clear liquid underneath the motor oil. There is no condesation on the sides of the test tubes. There are some white clumps at the bottom of the test tube. In test tube 1B the motor oil is glossy, golden and almost see through. The liquid underneath of the motor oil is white and clear. There is also white clumps at the bottom of this test tube. In test tube 1C the motor oil is also glossy, see through and golden coloured. The liquid underneath of the motor oil is also clear. There is no condensation in both test tube 1B and 1C. There is also white clumps at the bottom of the test tube. In test tube 1D there is no motor oil, but there is black/green/white mold on the surface of the liquid. There is also white/light brown clumps at the bottom of the test tubes. In test tube 1E there is a thin layer of white mold at the top. The liquid below the white mold is a light brown/orange, in this test tube there is a light brown clump at the bottom of the test tube. Test tube 1F also has a slightly thicker layer of white mold on top of the liquid. The liquid is a light-yellow colour. There are also some light brown clumps at the bottom of the test tube. Test tubes 1G-1I are almost identical. They all have condesation on the side of the test tubes. The colour of the motor oil is part cloudy yellow/orange/brown and cloudy light white/yellow. The liquid below the motor oil is a slightly yellow colour. These test tubes also have light brown/white clumps at the bottom. Test tubes 1J to 1L also seem

similar. The motor oil is slightly darker than test tubes 1I to 1G. It is only a cloudy golden syrup colour. The liquid below is a light yellow/green colour. This liquid is cloudy, and I cannot see through it. Test tubes 1M-1O are also very similar. All of the motor oil is see-through, glossy and looks very similar to when I first poured the motor oil inside except that there is some light white/grey lumps floating/clinging to the side of the test tubes. The liquid below is light yellow and cloudy and not see through. There are also white/light brown lumps at the bottom of the test tubes. All the "2" test tubes do not appear to have a huge change from today to yesterday.

Raw Observations of Phase 2

2021-02-28 to 2021-03-06

Day #1

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Shiny, glossy canola oil colored motor oil.	Clear	N/A
3C/D	N/A	Cloudy white	Brown
3E/F/G	Shiny, glossy canola oil colored motor oil.	Cloudy white	Brown
3H/I/J	Shiny, glossy canola oil colored motor oil.	Cloudy white	Brown
3K/L/M	Shiny, glossy canola oil colored motor oil.	Cloudy white	Brown

Day #2

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil.	Clear	N/A
3C/D	N/A	Slightly cloudy	There is a thin layer of white “goop” that is either the oxidized oil or lipids.
3E/F/G	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil.	Slightly cloudy	There is a thin layer of white “goop” that is either the oxidized oil or lipids.
3H/I/J	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil. All test tubes 3H-3M	Slightly cloudy	There is a slightly thicker layer of either oxidized oil or lipids at the bottom of the test tube covering the microbes. There are black dots at the bottom in the white oil (?) and it almost looks like cookies

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
	have a group of bubbles floating at the surface of the motor oil layer.		and cream ice cream. There is a thin layer of brown microbes in the middle and then more white broken down on top. The "goop" almost appears to be bubbling.
3K/L/M	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil. All test tubes 3H-3M have a group of bubbles floating at the surface of the motor oil layer.	Slightly cloudy	There is a slightly thicker layer of either oxidized oil or lipids at the bottom of the test tube covering the microbes. There are black dots at the bottom in the white oil (?) and it almost looks like cookies and cream ice cream. There is a thin layer of brown microbes in the middle and then more white broken down on top. The "goop" almost appears to be bubbling.

Day #3

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil.	Clear	N/A
3C/D	N/A	Cloudy white	Light Brown /white
3E/F/G	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil.	Clear	Light Brown /white
3H/I/J	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil. Hair floating in test tube. No more white bubbles.	Cloudy grey	Light Brown /white
3K/L/M	Slightly more dulled and a lighter color. There is a thin, uneven layer of bioremediated oil at the bottom of the layer of motor oil. All test tubes 3K-3M have a group of bubbles floating at the surface of the motor oil layer.	Cloudy grey	Light grey/white

Day #4

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Dulled light yellow/green/grey. The bioremediated oil is a cloudy grey/green color. The motor oil is a slightly dulled cloudy yellow color.	Clear	N/A
3C/D	N/A	Slightly cloudy grey	Light brown/grey appears grainy and almost "mush" like
3E/F/G	Dulled cloudy yellow at the bottom of the layer of motor oil, possible the bioremediated motor oil. The motor oil light shiny golden glossy color.	Clear	Slightly darker brown appears speckled and goo like
3H/I/J	Bioremediated oil is cloudy light yellow the remaining motor oil is glossy light yellow/golden and has air bubbles on the surface.	Cloudy grey	White, Grey & Brown
3K/L/M	There appears to be two different layers of bioremediated motor oil, one at the top of the motor oil and one at the bottom of the layer. This bioremediated motor oil is a cloudy light-yellow color. The motor oil is a glossy golden color.	Cloudy white/grey	White, Grey & Brown

Day #5

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Has turned mostly a light cloudy yellow most likely oxidized oil, because it cannot be lipids because there are no microbes in these test tubes.	Clear	N/A
3C/D	N/A	Slightly cloudy grey/white most likely from the fertilizer. There is a thin cloudier layer on the surface of the water, possibly mold?	White to brown going from lightest to darkest like ombre. The white is either lipids, oxidized oil, or possibly fertilizer if it has not dissolved
3E/F/G	Like egg white color hard to tell difference between bioremediated oil and remaining motor oil.	Clear	Similar brown ombre as test tubes 3C & 3D

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3H/I/J	Egg White color bioremediated oil and non-bioremediated oil now look very similar as there is not very much motor oil remaining.	Slightly cloudy	Microbes appear to be covered in a white substance either lipids, oxidized oil, or fertilizer (or a combination)
3K/L/M	Motor oil is the middle layer there appears to be two layers of bioremediated motor oil one at the bottom of the layer of oil and one at the top. The motor oil is a butter/egg white color.	Slightly cloudier – possibly because there is more fertilizer in these test tubes	More white substance than any other test tubes, cannot see microbes at all.

Day #6

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Around 80% of the motor oil appears to have oxidized, it has turned a very cloudy white. There is a small amount of non-oxidized oil remaining. Which is similar to Canola Oil	Clear	N/A
3C/D	N/A	Light cloudy grey/white	It just appears that there are just microbes at the bottom of the test tubes. I think that there are no lipids or oxidized oil because there is no oil and there is just microbes so the water cannot oxidized the oil and there are no oil for the microbes to bioremediate and breakdown into lipids.
3E/F/G	Around 80-85% of the motor oil is bioremediated by the microbes. The remaining 15-20% of motor oil is shiny/glossy butter color.	Clear	Brown and thick cloudy white substance – lipids or oxidized oil
3H/I/J	Around 60-70% of the motor oil appears to be bioremediated. The bioremediated oil is a light cloudy white/yellow, and the remaining motor oil is a glossy butter color.	Slightly cloudy white/grey	Mostly white substances at the bottom of the test tubes – lipids, or oxidized oil
3K/L/M	Around 90-95% of the motor oil appears to be bioremediated. The bioremediated oil is a light cloudy white/yellow, and the remaining motor oil is a glossy butter color.	Slightly cloudier white/grey	Mostly white substances at the bottom of the test tubes – lipids, or oxidized oil

Day #7

Test Tube	Color/Description of Oil	Color of Water	Color of microbes/liquid at bottom of test tube
3A/B	Around 90% of the motor oil now appears to be oxidized. The oxidized oil is a cloudy white/slightly yellow color. The remaining non-oxidized motor oil is still shiny and glossy.	Clear	N/A
3C/D	N/A	Slightly cloudy grey/white	Just brown microbes
3E/F/G	Around 90% of the motor oil appears to have been bioremediated. This bioremediated oil is cloudy light yellow. The remaining motor oil is still glossy, but the color appears to have lightened a bit. It appears that their also might be some oxidized oil as there are two layers one layer might be oxidized oil and the other layer bioremediated oil.	Clear	Mainly brown microbes small amount of a white substance.
3H/I/J	Around 80% of the oil now seems to be bioremediated. That oil is a cloudy light yellow/white. The remaining non-bioremediated oil is slightly glossy and more canola oil colored.	Slightly cloudy white/yellow	Pretty thick layer of oxidized oil, lipids, fertilizer (?) covering the microbes. I cannot see the microbes at all.
3K/L/M	All of the oil is now bioremediated. The bioremediated oil is all a cloudy butter color.	Slightly cloudy white/yellow	Even thicker layer of oxidized oil, lipids, fertilizer (?) covering the microbes. I cannot see the microbes at all.

Analysis Phase 1

Data and Calculations from Phase #1																					
Individual Test Tubes																					
Test Tube:	Amount of Distilled Water	Amount of Microbe Water	Amount of Oil	Amount of Fertilizer	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 1 to 14 Difference	Percent Oil Remaining	Percent Oil Reduction
	mL	mL	mL	g	Motor Oil amount in mm																
1A	55	0	2.5	2	6	6	6	6	6	6	6	6	6	6	6	6	6	5	1	83%	17%
1B	55	0	5	2	10	10	9	8	8	8	8	8	8	8	8	8	8	8	2	80%	20%
1C	55	0	8	2	14	14	14	13	13	13	13	13	13	13	12	12	12	12	2	86%	14%
1D	30	25	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no oil	no oil
1E	30	25	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no oil	no oil
1F	30	25	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no oil	no oil
1G	30	25	2.5	2	6	5	4	4	3	3	3	3	3	2	2	2	2	2	4	33%	67%
1H	30	25	2.5	2	6	5	4	4	3	3	3	3	3	2	2	2	2	2	4	33%	67%
1I	30	25	2.5	2	6	5	4	4	3	3	3	3	2.5	2	2	2	2	2	4	33%	67%
1J	30	25	5	2	10	9	8	8	7	7	7	7	8.5	6	5	4	4	3	7	30%	70%
1K	30	25	5	2	10	9	8	8	7	7	7	7	8.5	6	5	4	3	3	7	30%	70%
1L	30	25	5	2	10	9	8	8	7	7	7	7	8.5	6	5	4	3	3	7	30%	70%
1M	30	25	8	2	14	13	12	12	11	11	11	11	10	10	10	9	9	9	5	64%	36%
1N	30	25	8	2	14	13	12	12	11	11	11	11	10	10	9.5	9	9	9	5	64%	36%
1O	30	25	8	2	14	13	12	12	11	11	11	11	10	10	10	9	9	9	5	64%	36%
2A	55	0	5	1	8	8	8	8	8	7	6	6	6	5	3	3	5	5	3	63%	38%
2B	55	0	5	2	8	8	8	8	8	7	6	6	6.5	5	4	4	4	5	3	63%	38%
2C	55	0	5	3	8	8	8	8	8	7	6	6	6.5	5	5	5	5	5	3	63%	38%
2D	30	25	5	0	8	7	6	6	6	6	6	6	5	4	3	3	2	2	6	25%	75%
2E	30	25	5	0	8	7	6.5	6	6	6	6	6	5	4	3	3	2	2	6	25%	75%
2F	30	25	5	0	8	7	6	6	6	6	6	6	5	4	3	3	2	2	6	25%	75%
2G	30	25	5	1	8	7	7	7	6	6	6	6	7	6	3	3	3	3	5	38%	63%
2H	30	25	5	1	8	7	7	7	6	6	6	6	7	6	3	3	3	3	5	38%	63%
2I	30	25	5	1	8	7	7	7	6	6	6	6	7	6	3	3	3	3	5	38%	63%
2J	30	25	5	2	8	7	7	7	6	6	6	6	6	5	4	3	3	3	5	38%	63%
2K	30	25	5	2	8	7	7	7	6	6	6	6	6	5	4	3	3	3	5	38%	63%
2L	30	25	5	2	8	7	7	7	6	6	6	6	6	5	4	3	3	3	5	38%	63%
2M	30	25	5	3	8	7	7	7	6	6	6	6	6	4	3	3	2	2	6	25%	75%
2N	30	25	5	3	8	7	7	7	6	6	6	6	6	4	3	2	2	2	6	25%	75%
2O	30	25	5	3	8	7	7	7	6	6	6	6	5	4	3	2	2	2	6	25%	75%

Data and Calculations from Phase #1 Average by Test Tube Type																	
Test Tube:	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 1 to 14 Difference (mm)	Percent Oil Remaining	Percent Oil Reduction
	Motor Oil amount in mm																
1A-Control-No bugs-6mm oil-2g nutrients	6	6	6	6	6	6	6	6	6	6	6	6	6	5	1	83%	17%
1B-Control-No bugs-10mm oil-2g nutrients	10	10	9	8	8	8	8	8	8	8	8	8	8	8	2	80%	20%
IC-Control-No bugs-14mm oil-2g nutrients	14	14	14	13	13	13	13	13	13	13	12	12	12	12	2	86%	14%
1D/E/F-Control-No oil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No Oil	No Oil
1G/H/I-6mm oil-2g nutrients	6	5	4	4	3	3	3	3	2.8	2	2	2	2	2	4	33%	67%
1J/K/L-10mm oil-2g nutrients	10	9	8	8	7	7	7	7	8.5	6	5	4	3.3	3	7	30%	70%
1M/N/O-14mm oil-2g nutrients	14	13	12	12	11	11	11	11	10	10	9.8	9	9	9	5	64%	36%
2A-Control-No bugs-8mm oil-1g nutrients	8	8	8	8	8	7	6	6	6	5	3	3	5	5	3	63%	38%
2B-Control-No bugs-8mm oil-2g nutrients	8	8	8	8	8	7	6	6	6.5	5	4	4	4	5	3	63%	38%
2C-Control-No bugs-8mm oil-3g nutrients	8	8	8	8	8	7	6	6	6.5	5	5	5	5	5	3	63%	38%
2D/E/F-Control-8mm oil-No nutrients	8	7	6.2	6	6	6	6	6	5	4	3	3	2	2	6	25%	75%
2G/H/I-8mm oil-1g nutrients	8	7	7	7	6	6	6	6	7	6	3	3	3	3	5	38%	63%
2J/K/L-8mm oil-2g nutrients	8	7	7	7	6	6	6	6	6	5	4	3	3	3	5	38%	63%
2M/N/O-8mm oil-3g nutrients	8	7	7	7	6	6	6	6	5.7	4	3	2.3	2	2	6	25%	75%

Data and Calculations from Phase #1 Oil Reduction - Average by Test Tube Type														
Test Tube:	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
	Motor Oil amount in mm													
1A-Control-No bugs-6mm oil-2g nutrients	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1B-Control-No bugs-10mm oil-2g nutrients	0	0	1	2	2	2	2	2	2	2	2	2	2	2
IC-Control-No bugs-14mm oil-2g nutrients	0	0	0	1	1	1	1	1	1	1	2	2	2	2
1D/E/F-Control-No oil	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1G/H/I-6mm oil-2g nutrients	0	1	2	2	3	3	3	3	3.2	4	4	4	4	4
1J/K/L-10mm oil-2g nutrients	0	1	2	2	3	3	3	3	1.5	4	5	6	6.7	7
1M/N/O-14mm oil-2g nutrients	0	1	2	2	3	3	3	3	4	4	4.2	5	5	5
2A-Control-No bugs-8mm oil-1g nutrients	0	0	0	0	0	1	2	2	2	3	5	5	3	3
2B-Control-No bugs-8mm oil-2g nutrients	0	0	0	0	0	1	2	2	1.5	3	4	4	4	3
2C-Control-No bugs-8mm oil-3g nutrients	0	0	0	0	0	1	2	2	1.5	3	3	3	3	3
2D/E/F-Control-8mm oil-No nutrients	0	1	1.8	2	2	2	2	2	3	4	5	5	6	6
2G/H/I-8mm oil-1g nutrients	0	1	1	1	2	2	2	2	1	2	5	5	5	5
2J/K/L-8mm oil-2g nutrients	0	1	1	1	2	2	2	2	2	3	4	5	5	5
2M/N/O-8mm oil-3g nutrients	0	1	1	1	2	2	2	2	2.3	4	5	5.7	6	6

Analysis Phase 2

Data and Calculations from Phase #2 Individual Test Tubes														
Test Tube	Amount of POND Water	Amount of Microbe Water	Amount of Oil	Amount of Fertilizer	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 1 to7 Difference (mm)	Percent Oil Remaining	Percent Oil Reduction
	mL	mL	mL	g	Motor Oil Amount in mm									
3A	55	0	2.5	0	5	5	5	5	5	5	5	0	100%	0%
3B	55	0	2.5	0	5	5	5	5	5	5	5	0	100%	0%
3C	30	25	0	0	0	0	0	0	0	0	0	0	no oil	no oil
3D	30	25	0	0	0	0	0	0	0	0	0	0	no oil	no oil
3E	30	25	2.5	0	5	3	2	2	2	1	0.5	4.5	10%	90%
3F	30	25	2.5	0	5	3	2	2	2	1	0.5	4.5	10%	90%
3G	30	25	2.5	0	5	3	2	2	1.75	1	0.5	4.5	10%	90%
3H	30	25	2.5	1.5	5	4	4	3	2.5	2	1	4	20%	80%
3I	30	25	2.5	1.5	5	4	3	3	2.5	2	1	4	20%	80%
3J	30	25	2.5	1.5	5	4	3	3	2.5	2	1	4	20%	80%
3K	30	25	2.5	3	5	4	3	2	1	0.5	0	5	0%	100%
3L	30	25	2.5	3	5	4	3	2	1	0.5	0	5	0%	100%
3M	30	25	2.5	3	5	3.5	3	2	1	0.5	0	5	0%	100%

Data and Calculations from Phase #2 Average by Test Tube Type										
Test Tube Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 1 to7 Difference (mm)	Percent Oil Remaining	Percent Oil Reduction
	Motor Oil Amount in mm									
3A/3B-Control-No bugs-5mm oil-no nutrients	5	5	5	5	5	5	5	0	100%	0%
3C/3D-Control-No oil-no nutrients	0	0	0	0	0	0	0	0	No oil	No oil
3E/3F/3G-5mm oil-no nutrients	5	3	2	2	2	1	0.5	4.5	10%	90%
3H/3I/3J-5mm oil-1.5g nutrients	5	4	3	3	3	2	1	4	20%	80%
3K/3L/3M-5mm oil-3g nutrients	5	4	3	2	1	1	0	5	0%	100%

Phase #1 Data Analysis

In Table 1 the oil column height measurement data was averaged for the three test tubes in each group. The data from Table 1 was used to create Graph 1 (Group 1) and Graph 2 (Group 2). These graphs show how the amount of non-bioremediated oil column height changed over the 14-day period. In Group 1 the amount of motor oil was varied so there were various starting oil column heights. In Group 2 the oil column height was the same for all tests at the start. It is easier to identify which test tube groupings had the most bioremediation occur.

Table 2 shows the average reduction in oil column height over the 14-day period. Graph 3 (Group 1) and Graph 4 (Group 2) show this data visually. This was done to visually see the oil reduction over time since some of the test started at different oil heights at the start.

Table 3 shows the average oil column height difference from Day 1 to 14, the percentage of oil remaining and the percentage of oil bioremediated. This is visually represented in Graph 5 and 6. Graph 5 shows the total oil column height reduction over the 14 days for both Group 1 and 2. Graph 6 shows the oil column height percentage reduction which is the percent of oil column that was bioremediated over two weeks for both groups.

The Phase #1 Group 1 (varying oil column – all 2g nutrients at start) data shows:

- Test 1J/K/L with the medium oil column (10mm) had the most oil column reduction (7mm) which was also the biggest percent reduction (70%) for Group 1.
- Test 1M/N/O with the biggest oil column (14mm) had the second largest oil column reduction (5mm) but this only represents 36% oil column reduction which was the lowest out of the non-control tests in Group 1.
- Test 1G/H/I with the smallest oil column (6mm) had the smallest oil column reduction (4mm) outside of the controls. This is 67% of oil column reduction which is the second largest percent reduction but only 3% lower than the 10mm start test.
- The Control tests with no bugs showed 14-20% oil column reduction. This is possibly oxidized oil rather than bioremediated oil but when measurements were collected in Phase #1 oxidized oil was not yet understood.
- The time from 7 to 14 days increased the amount of bioremediated oil for all non-control test tubes. This is important because in Phase 2 the experiment was only 7 days due to time restriction.

The Phase #1 Group 2 (varying nutrients – all 8mm oil column at start) data shows:

- Test 2M/N/O with the most nutrients (3g nutrients) and Control 2D/E/F (no nutrients) had the largest oil column reduction after 14 days. They both reduced by 6 mm (75%). This percent reduction is 5% higher than the highest percent reduction from Group 1.
- Test 2G/H/I (1g nutrients) and Test 2J/K/L (2g nutrients) had the second largest oil column reduction after 14 days. They both reduced by 5 mm (63% reduction).
- The other Control 2A/B/C with no microbes and variations of nutrients (1g/2g/3g) all decreased by 3mm after 14 days. This is most likely oxidized oil.
- Some measurements went up and down which is most likely from human error or the shaking/stirring process.

Phase #2 (Pond Water) Data Analysis

In Table 4 the oil column height measurement data was averaged for the three test tubes in each group. The data from Table 4 was used to create Graph 7 which show how the amount of non-bioremediated oil column height changed over the 7 day time period.

Table 5 shows the average oil column height difference from Day 1 to 7, the percentage of oil remaining and the percentage of oil bioremediated. This is visually represented in Graph 8 and 9. Graph 8 shows the total oil column height reduction over the 7 days. Graph 6 shows the oil column height percentage reduction which is the percent of oil column that was bioremediated over one week.

The data from Phase #2 (Pond water; varying nutrients; 5mm oil column at start) shows:

- Test 3K/L/M with the most nutrients (3g) showed an oil column decrease of 5mm (100%) over the course of 7 days.

- Test 3E/F/G with no nutrients showed an oil column decrease of 4.5mm (90%) over the course of 7 days.
- Test 3H/I/J with the middle amount of nutrients (1.5g) showed an oil column decrease of 4mm (80%) over 7 days.
- All of these tests were within 1mm of reduction after 7 days.

Conclusions

General project conclusions are:

- The highest amounts of oil had a lower percent reduction. This may be because the microbes needed more time and/or because they could not reach the surface of the oil column easily. This may indicate large oil volumes require a longer time period, may require source removal to have further success and/or may require additional mixing.
- With medium and small amounts of oil present a significant amount of bioremediation can occur in 7-14 days.
- Nutrient addition of 3g into pond water with a 5mm oil column does not have a significant effect on the amount of bioremediation over 7 days. The type and ratio of fertilizer used may have affected these results. This may also indicate the pond water has nutrients present.
- Some degradation of oil occurs even without the addition of microbes to the samples, particularly in pond water.
- It was challenging to visually assess the difference between non-bioremediated oil, bioremediated oil, oxidized oil and/or lipids in the test tube experiment.

Review of My Hypothesis Compared to Results

Phase #1 – Groups 1 – Varying Amounts of Nutrients

- **Lowest Motor Oil (2.5mL):** I think this amount of motor oil will be completely bioremediated within the two-week period. From my research, bioremediation is most successful when as much source contaminant and free oil is removed. This hypothesis was incorrect because there was still 2mm of motor oil remaining after the two-week experiment.
- **Medium Motor Oil (5mL):** I think this amount of motor oil will only be partially bioremediated by the time the two-week period is up. The thicker oil column will mean the bioremediation will take longer because there is more oil to consume and because it is harder for the microbes to access the top of the oil layer. This hypothesis was correct because although not all of the motor oil was bioremediated all except for 3mm were bioremediated and/or oxidized.
- **Highest Motor Oil (8mL):** I think this amount of motor oil will also only be partially bioremediated and even more oil will be remaining than in the medium test. This hypothesis was also correct because only 5mm of the 14mm were bioremediated.

Phase #1 – Group 2 – Varying Amounts of Nutrients

- **Lowest Nutrients (1g)**: I think that this amount of nutrients will not do much to stimulate microbial growth and contaminant removal and would have similar results to the controls with zero nutrients. This hypothesis was incorrect because the fertilizer might have stimulated bioremediation these test tubes started with 8mm of motor oil and dropped to 3mm over the course of 2 weeks.

- **Medium Nutrients (2g)**: I think that this amount of nutrients will have the most bioremediation because I think this amount will not be toxic to the microbes but will still stimulate bioremediation and microbe reproduction. This hypothesis was also incorrect because this test tube did not have the most bioremediation. These test tubes also went from 8mm to 3mm over the course of 2 weeks.
- **Highest Nutrients (3g)**: From my research I know that high amounts of nutrients may kill the microbes but I am not sure what ratio will be toxic. This nutrient is the highest of my tests so I think it is most likely to have toxic effects. My hypothesis was also incorrect because this amount of nutrients did not prove to be toxic to the microbes. These test tubes also had the most bioremediation occur and went from 8mm to 2mm over the course of 2 weeks.

Phase #2 – Pond Water – Varying Amounts of Nutrients

- **No Nutrients (0g)**: I think that no nutrients will still have a high level of bioremediation based on my phase 1 test. My hypothesis was correct because these test tubes went from 5mm to 0.5mm in 7 days which was the second most amount of bioremediation.
- **Medium Nutrients (1.5g)**: I think that this amount of nutrients will not have the least amount of bioremediation based on results from my phase 1 test. This hypothesis was correct because these test tubes did have the least amount of bioremediation occur, they went form 5mm to 1mm in 7 days.
- **Highest Nutrients (3g)**: I think that this amount of nutrients will have the highest level of bioremediation. Phase 1 showed that this amount of nutrients is not toxic and stimulates microbial reproduction and bioremediation. Please refer to data analysis tab for details on data and how it was analyzed. This hypothesis was correct because this amount of fertilizer was not toxic and stimulated microbial reproduction and bioremediation. These test tubes had the most bioremediation occur and went form 5mm to 0mm in 7 days.

Next Steps and Application to the Real World

Next Steps and General Application

Generally, this project is important because oil spills happen in both salt and fresh water although saltwater seems to have more studies done on it. It is important that ideal quantities of different items that help speed up bioremediation in freshwater are understood.

- Additional research into the type and ratio of nutrients is needed. Based on conclusions so far, the fertilizer selected does not have a significant effect on bioremediation. Fertilizer can affect the flora and fauna of the site so the risk to the site with adding chemical fertilizer may outway the benefits to the bioremediation process of adding fertilizer. More research and testing of natural nutrient sources would be helpful for freshwater use in the real world.
- Tests with larger amounts of oil indicate that longer time periods eventually have success. It would be interesting to test for longer periods of time.
- It would be interesting to do another test using pond water but in larger containers such as buckets to see how the larger sample size changes the test. Thinking about mixing and making sure oxygen is being provided to the microbes is also important.
- Further tests could study how other variables may affect the rate of biodegradation (such as pH and temperature) although there is quite a lot of information available indicating the best range for these variables. The type and amount of nutrients has less research available, particularly for freshwater, which is why it was selected for this experiment.

- The test showed the importance of removing excess oil prior to starting bioremediation of freshwater. Some ways to remove excess oil on freshwater before starting bioremediation include skimmers, boom arms, absorbent material, and even burning.
- It would be interesting to source products that are sold commercially for real-world biodegradation application and test the products on freshwater oil spills as the microbes that I used were sold in small quantities for laboratory use. I was able to find a product sold by Acklands Granger in Calgary that apparently contains oil-eating microbes stored in a dried bentonite clay mix (Ultra-Archaea sold by Ultratech International). The directions for this product indicate it can be applied to oil-contaminated areas and the microbes will digest the pollutants.

Bragg Creek Pond Application

The reason I became interested in bioremediation of hydrocarbons in freshwater is because my Grandparent's had a bobcat spill diesel in their pond in Bragg Creek over Christmas this year. We helped research ways to clean up the spill and this led to my project idea. I could not test diesel specifically as it is too smelly to have in our house which is why I picked motor oil. Once the ice melts on the pond in the spring I would like to go out and study the spill area to see if there is any indication of remaining diesel. If there is still diesel present I am hoping I can take what I learned and help clean up the pond.

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Scanned Pages from Planning and Research in my Notebook

2021-01-17

Project Division
Life and Health Sciences

Topic
- Earth Science
- Environmental Science
- Microbial Biology
- Pollution

2021-01-20
Testable Question
Does

Materials List
Microbes -> the freeze dried powdered microbes that I ordered were from the Pseudomonas, Flavobacterium, Arthobacter and Azobacter generas (genus) - general grouping.

2020-01-2020

What is biodegradation?

"biodegradation is the process in which organic Compounds are degraded or broken down by the microorganisms." (The Biology Notes)

definition of degraded

"The reduction of a chemical compound to one less complex, as by splitting off one or more groups." (Biology Online)

definition of Metabolic

- Often refered to the "breakdown of food and its transformation into energy." (RxList)

What is Biostimulation

1. "Addition of materials to enhance the growth and activity of indigenous microorganisms in an contaminated site (IGI Global)
2. "The modification of the environment to stimulate organisms that are capable of bioremediation." (IGI Global)

Aerobic Bacteria

"Bacteria that can grow and live when oxygen is present." (UCSF Health)

Aerobic Biodegradation

"Is done by aerobic microorganisms when an adequate supply of oxygen is available. It is a rapid method that degrades

2021-01-20

the contaminants completely." (The Biology Notes)

Anaerobic Biodegradation

"Takes place in the absence of oxygen. It's pathway has four major steps:" (The biology notes)

1. Hydrolysis
2. Acidogenesis
3. Acetogenesis
4. Methanogenesis

Bioremediation

"Bioremediation is the process that uses microorganisms or plants to clean polluted environments. Naturally ocurring or introduced organisms (especially microorganism (which break down environmental pollutants) can be used in bio remediation." (The Biology Notes)

In Situ Bioremediation

"Contaminants are broken down at the site of the originated in in-situ bioremediation." (The Biology Notes)

"You allow bioremediation to take place while leaving the soil or water in its natural location. (study.com)

Ex-situ bioremediation

Contaminants that are treated out of the Contamination site is known as ex-situ bioremediation.

"Ex-situ bioremediation describes a process where contaminated soil or water is removed from the environment by biological organisms. Ex-situ bioremediation can use bioreactors and added nutrients to speed up the breakdown of environmental pollutants. (study.com)

2021-01-20

Bioreactors

"An apparatus for growing organisms (yeast, bacteria, or animal cells) under controlled conditions." (Introduction to Chemical and Biological Engineering)

Apparatus

a. "a set of materials or equipment designed for a particular use." (Merriam Webster)

Anatomical

"Relating to anatomy or the body structure of organisms." (Merriam Webster)

2021-01-21

Cytology

"The branch of biology concerned with the structure and function of plant and animal cells." (Oxford Languages)

How do added nutrients affect the microbes in bioremediation and biodegredation?

Am I going to shake test tube to represent waves?

2021-01-24

Crude Oil

- Crude oil is unrefined petroleum
- "Crude oil, also known as petroleum, is an energy-rich liquid consisting of mainly hydrocarbons." (Government of Canada)

Petroleum

"Also called crude oil, is a fossil fuel. Like coal and natural gas, petroleum was formed from the remains of ancient marine organisms, such as plants, algae, and bacteria. Over millions of years of intense heat and pressure, these organic remains (fossils) transformed into carbon-rich substances which we rely on as raw materials for fuel and a wide variety of products. (National Geographic)

Materials List

- 10grams oil-eating microbes (freezedried powder)
- Gloves
- Goggles
- Test tubes
- Crude oil
- Nutrients

Might Need

- lids for test tubes
- Scale (grams) that can measure 0.5 g
- Density indicator?
- Bulb for pipette
- Incubator

2021 - 01 - 24

Control

- Type and size of testtubes
- Type of microbes
- Same magnet size
- Same process to culture microbes
- Same type of unused motor oil
- Same temperature
- Same number of "shakes" per day
- pH
- Same amount and type of distilled water
-

Manipulated

Amount of nutrients (fertilizer) 0.5g, 1g, 3g

Amount of motor oil? (2%, 5%, 10%

dependent

Increase and/or decrease of degredation rates

Does changing amounts of nutrients and crude oil affect the amount of biodegredation and bioremediation

2021-01-24

Procedure

1. Wash hands
2. Wash equipment with soap and water & triple rinse
3. Put on nitrile gloves, goggles, apron and tie back hair
4. Label lids of testubes with experiment number and what will go inside - example experiment #1, water, 2% oil, microbes, and nutrients
5. Add 0.5g of Oil eating microbe (OEM) powder to 250mL of tap water in a 500mL
6. Put lid on flask
7. Place flask on stirer set at 200rpm using medium stir bar (roomtemperature) let stir for 16-20 hours
8. Pour 40mL of distilled water into 30 viles
10. Pour 5mL 2.5mL of motor oil into 15 test tubes
9. Dissolve 2g of nutrients into the other 15 testubes
11. Pour 2% motor oil into 3 of the test tubes with nutrients
12. Pour 5% motor oil into 3 of the test tubes with nutrients
13. Pour 10% motor oil into 3 of the test tubes with nutrients
14. Dissolve 0.5 g nutrients into 3 of the test tubes with oil
15. Dissolve 1g nutrients into 3 of the test tubes with oil
16. Dissolve 3g nutrients into 3 of the test tubes with oil
17. To 12 of the test tubes with nutrient variations add 25mL of microbes
18. To 12 of the test tube with motor oil variations add 25mL of microbes

* 3 testubes from the nutrient variation category and 3 testubes from the oil variation category should only have 40mL of distilled water and 2.5mL of oil / 2g nutrients

2021-01-27

Today I started a test experiment & I washed my hands, put on gloves, googles. Then I zeroed the scale with our container on it. Next I fill a 500 mL Flask with 250mL of cold water. Then I carefully measured out 0.5g of microbes in the container using the pink plastic spoon. Next I poured the microbes into the water in the flask. I then place a magnet (the one that came with the magnetic stirrir) inside of the flask. Then I put a size 7 lid on the Flask and placed the Flask on the stirrir. I set the speed of the stirrir to approximately 200rpm (rotations per minute) although it may not be exact. Now I am going to let the microbes stir for 16hours.

I started the stir at 8:45pm

Observations

After only stirring the mixture for a few minutes all of the liquid appears to be a light tan colour with darker specks swirling around inside.

2021-01-28

CYSF 2021

Observations

The microbes have been stirred at approximately 200rpm for about 23 hours now

- I have noticed that there has been a slight increase in the amount of liquid. The liquid is now above the 250mL line. This could mean that in the "re-viving" of the microbes they have expanded, or the microbes are happy and have already started breding. or because the liqui was in a whirl pool
- The magnet has been quite noisy and shifts around banging into the sides of the flask
- With the magnet, moving around it creats a swirl / tornado in the water

The liquid is still a tan colour and there are still dark brown (•?) coloured specks floating around
- This could be the microbes

When I turned off the stirrir the liquid went back down to 250mL
The liquid is now also cloudy and you cannot see through it

2021-01-28

After I revived the microbes I pour 25mL of bacteria into 10 different test tubes. I labeled 5 1a, 1b, 1c, 1d, & 1e. 1A contained 40mL of distilled water 25mL bacterial, 1g fertilizer & 1mL of oil. 1b contained 40mL of distilled water and 25mL bacteria and 1mL of oil. 1b did not contain any fertilizer. 1c contained 40mL distilled water, 25 mL bacteria, 1g fertilizer and 2.5 mL oil

2021 - 02 - 04

Materials List:

- Gloves
- Goggles
- Distilled water
- Water
- 10g Freeze dried microbes
- Motor oil
- Fertilizer
- pipettes
- Test tubes
- Lids
- 2 small containers
- Heating mat
- Magnetic Stirrir
- Beakers

2021 - 02 - 04

Testable Question

How does changing amounts of fertilizer (nutrients) and motor oil in an controlled oil spill affect the speed /amount of oil that is ~~degraded~~ bioremediated by added microbes?

"The key difference between biodegradation and bioremediation is that biodegradation is a natural process that occurs in the environment, and bioremediation is an engineered technique applied by humans to clean the environment.

* Both processes involve microorganisms

How do oil spills impact marine life

I have to fill out Ethics and Due Care Form 2A by tonight

2021 - 02 - 06

After doing a test experiment I have concluded that 1mL & 2.5mL of oil is not enough oil to be able to see a difference. Also the 40mL of distilled water seemed to much because I plan to swirl the test tube around once daily to represent movement like in a river, lake or ocean. Also with no distilled water their appeared to be less bioremediation

2021 - 02-06

Today I am reviving the bacteria. During the test I used 250mL of water and 0.5g of OEM powder which was enough for 2.5 testtubes. For the experiment I need enough liquid for 30 test tubes with 25mL per test tube. On the magnetic stirrir I will have a 1,000mL beaker with 1,000 oz of water that has sat out for 3 hours to let the chlorene inside disipate. I will have 2g of the OEM powder in the beaker. I will make a cover for the beaker out of tinfoil to prevent spilling. I will use the magnetic that is 4cm long. The microbes will be stirred for 24 - 26 hours depending on how cloudy the mixture is.