Project Learning Tree®

Exploring Environmental Issues: Biotechnology Supplement

Introduction to Activity 2

Bioremediation

Background

- It this activity you will explore common types of environmental contamination
- You will then explore ways toxic or hazardous waste can be cleaned from the soil and water in an environment

What is bioremediation?

- It is the process of using living organisms to absorb nutrients in order to restore an ecosystem to its natural condition

When does bioremediation happen?

- Often remediation is unplanned - such as the breakdown of chemicals that have run off of roads, agricultural fields, or seeped from industrial storage tanks

- Bioremediation can also be planned – such as the use of beets to draw salt from agricultural fields

How does bioremediation happen?

Living organisms can be used to:

- Trap toxins or hold contaminants in place or to simply slow the migration of toxins through soil or ground water
- Decompose chemicals by metabolizing toxic molecules into inert forms
- Hyperaccumulate or sequester a toxic substance for collection by humans

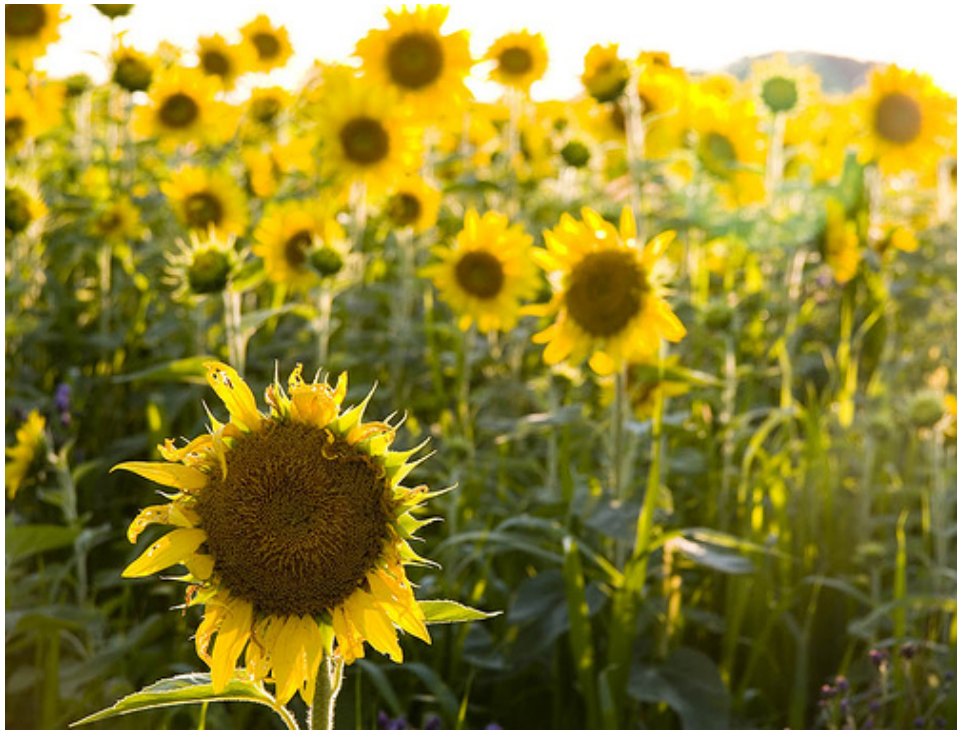
Where do we see bioremediation taking place?

- Compost piles of vegetation or manure
- Sewage treatment
- Mining tailing piles and the surrounding area
- Oil spills and water clean up
- Brownfields or industrial storage facilities
- Overflows, waste spills and accidents

Plants can perform bioremediation through metabolism

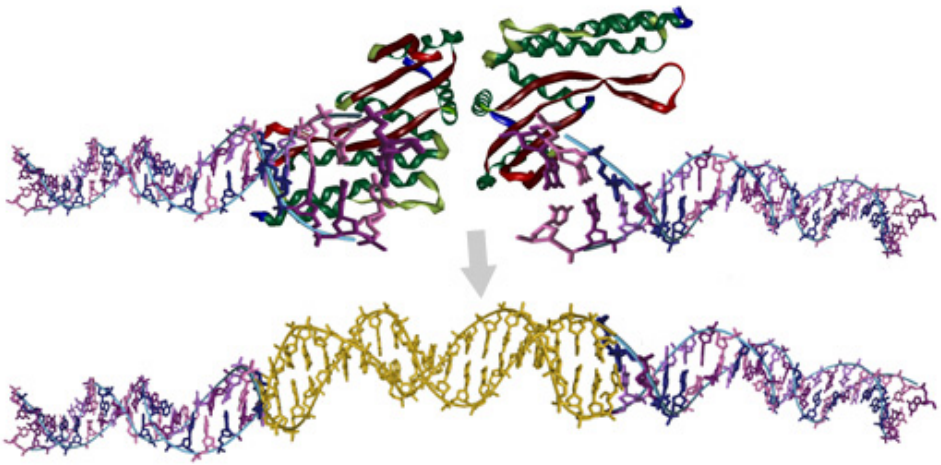
- Plants can draw chemicals in through their roots to metabolize toxins and release less toxic forms of that substance through their leaves
- For example, poplar species such as willow and cottonwood trees, can absorb toxic forms of mercury from the soil and release a less toxic vaporized compound into the air

Plants can perform bioremediation using hyperaccumulation



- Plants can absorb toxins through their roots and store them in their tissues where humans can collect them
- For example, sunflowers can be used to hyperaccumulate radioactive strontium from the soil and water in sites where a nuclear disaster has occurred

How do plants help with bioremediation?



- The plants that are used may be naturally occurring species or species that are genetically engineered to absorb a specific toxin
- For instance a scientist may insert a gene to metabolize a particular industrial chemical in a fast growing plant with deep roots to clean up a groundwater contamination

How do organisms help with bioremediation?

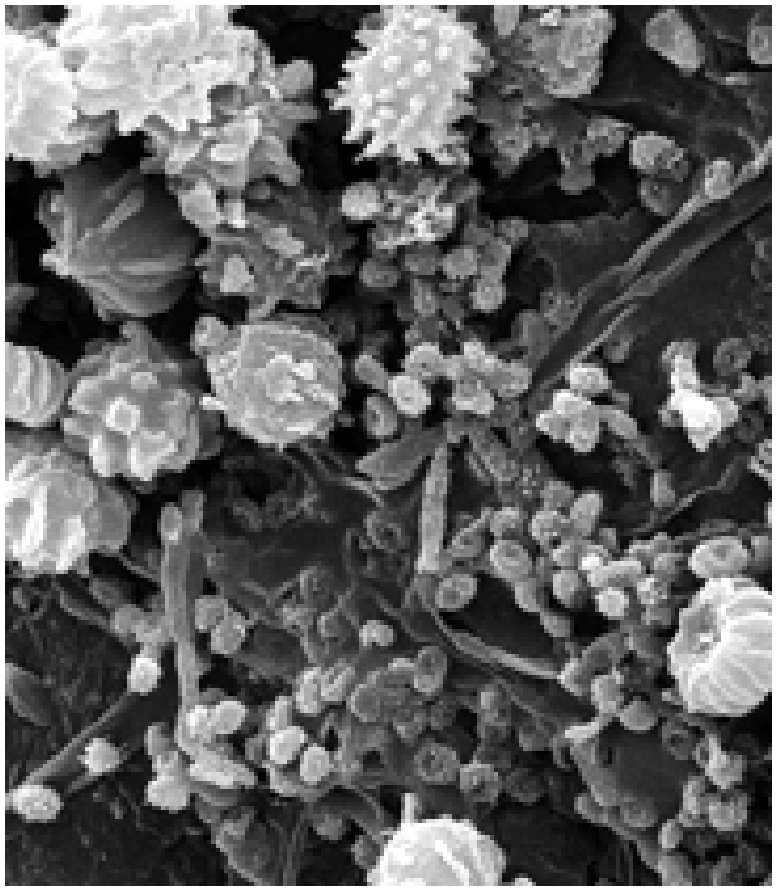
- Local organisms in the soil, the surface or groundwater can metabolize or sequester toxins

How does bioaugmentation help with bioremediation?

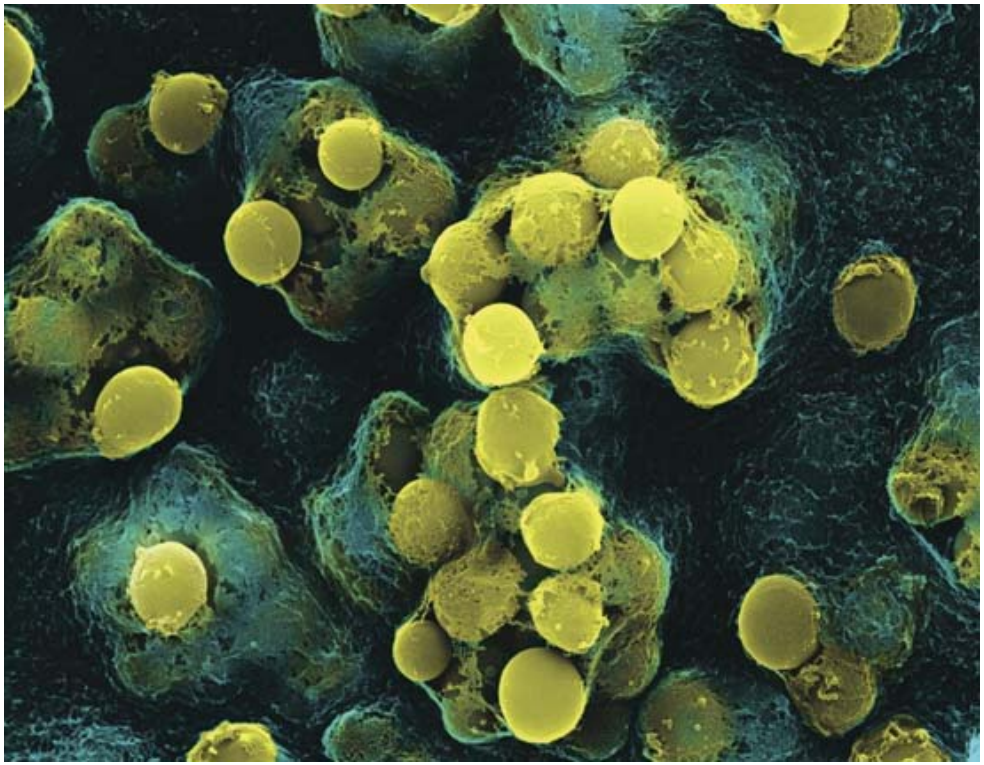
- Microorganisms, algae, worms, snails and other organisms can be added to an area to decompose sewage and farm waste by encouraging the metabolic breakdown of the waste
- When non-native microorganisms are introduced to an area to help clean up a specific toxin the process is called bioaugmentation

How does biostimulation help with bioremediation?

- Humans can encourage the microorganisms to grow and multiply using biostimulation
- During biostimulation limiting factors such as oxygen, iron, magnesium, nitrate, sulfate or carbon dioxide may be added to the soil to foster microorganism growth



How do microorganisms help with bioremediation?



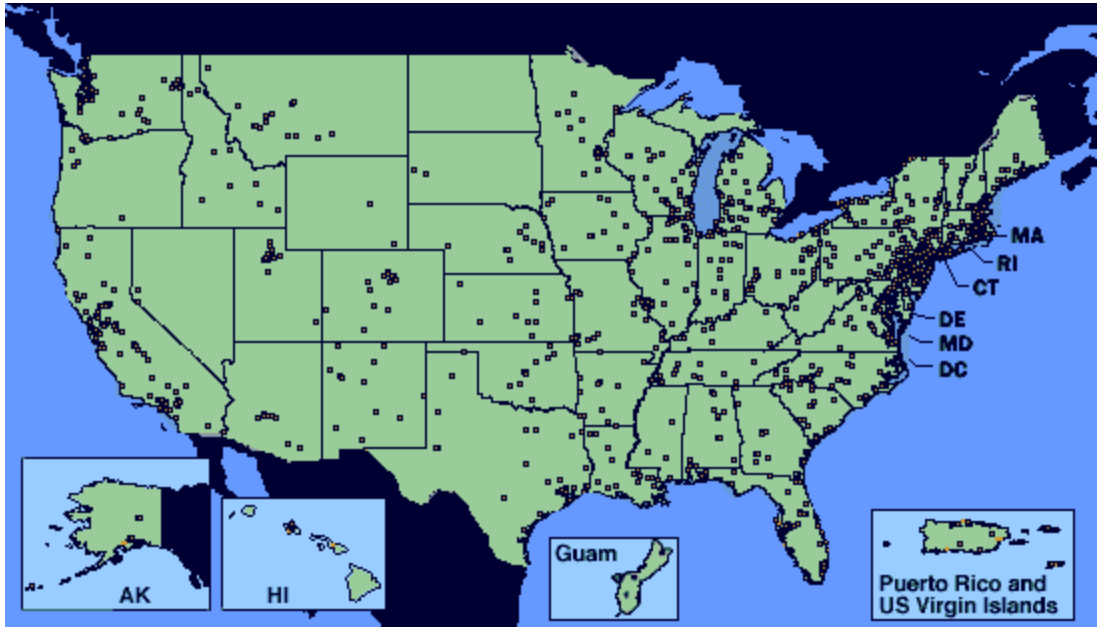
- Any introduced microorganism may be naturally occurring or genetically engineered

Getting help with bioremediation

- Determine the contaminant that needs to be cleaned up
- Find an organism that can absorb or break down that contaminant
- Harvest the genes that allow that organism to clean up contaminant and then place those genes in another organism that is better suited to the contaminated environment
- Or, use the organism directly in that environment and help it grow and reproduce using biostimulation

Part A: Superfund Sites

- What is a contaminated environment?
- Where do they occur?
- What types of pollution are there?



Superfund Sites

- What is being done to restore these areas to their natural condition?
- What are the methods of clean up?
- What is a typical time frame for the remediation?
- What will be the final results?

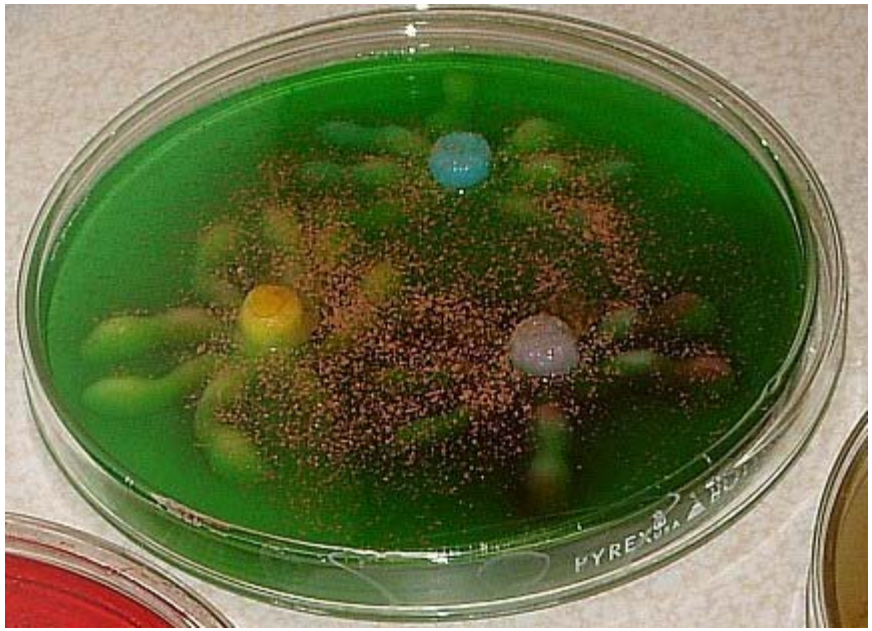
Activity: Superfund Sites

- During this activity you will learn about places in the United States that are poisoned by chemicals in the soil or water.
- You will research your region to find out where the nearest contaminated sites are located.
- You will use the Internet to learn about the chemicals that are contaminating a particular site and to find information about what is being done to clean up these sites.
- You will present what you have found to your peers orally and field any questions they have.

Reflection questions

- Did the number of Superfund sites surprise you?
- What did you think about the environmental health of your region before you used the Internet?
- What do you think of the environmental health of your region now that you have completed your research?
- What will you do with what you now know?

Part B: Bacteria Lab Experiments



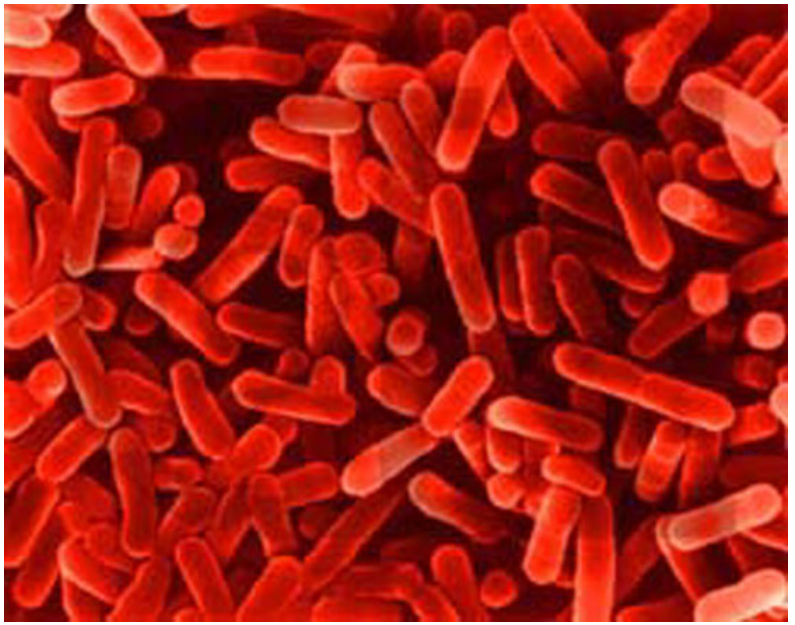
- In Part B you will learn about the organisms that can be used to clean up environmental disasters
- By fostering the growth of bacteria and other microorganism, toxins can be broken down to limit damage in an ecosystem
- How do we nurture these organisms to provide hazardous waste cleanup?

How do you define the biotic range of an organism?

- How do the specific abiotic (temperature, pH, moisture, oxygen, sunlight, etc.) needs of organisms differ?
- Contrast the limiting factors of a desert compared to a rainforest to point out how the abiotic factors of an ecosystem can select against the presence of particular organisms in that ecosystem.
- How do the specific biotic (type of food, symbiotic relationships, etc.) needs of organisms differ?
- Contrast the niches of a hawk and a duck to compare how abiotic and biotic factors play a role in determining an organism's role in an ecosystem.

How do the abiotic and biotic needs of bacteria differ?

- All bacteria, like other organisms, need a source of carbon for energy – but not all bacteria use the same carbon sources.
- All bacteria perform cell respiration – but not all bacteria need oxygen in order to perform this function.
- All bacteria need a substrate to live upon – but some bacteria can move during their lifetime to find a new substrate.



Bacteria can be used to clean up toxins in the environment

What characteristics would be desirable in a bacterium that is used to clean up

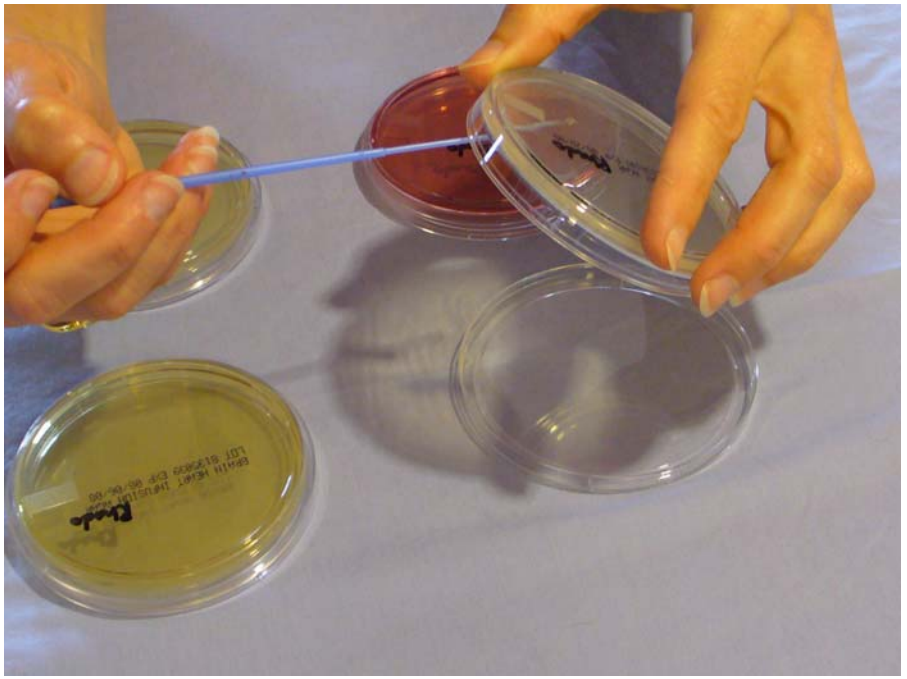
- acids that are leaking from a mining site into the soil?
- waste chemicals from an industrial plant's storage facility?
- an oil spill on the surface of the ocean?

Activity: Bacteria Lab Experiment

- During this activity you will try to grow bacteria on agar habitats that offer different compositions of nutrients.
- Some bacteria strains will grow on several different types of nutrient media, others are pickier and will not survive.
- To make sure you are only growing one type of bacteria on a particular media plate, you will need to use sterile technique (the next few slides will teach you this procedure).

Sterile technique do's and don't's

- Never leave the media plates open on the table.
- To avoid contamination of the media plate by fungal spores in the air, keep the plates turned upside down (with the media on the top part of the Petrie dish) and only open them a crack when it is time to slide an inoculating loop underneath.



Sterile technique do’s and don’t’s

- Do not open the inoculating loops until you are ready to use them.
- Never touch the inoculating loops to any surface other than the bacteria source or the media plates where they are being spread.
- Only touch your hands to the middle section of the loops.
- Dispose of the loops in the trash immediately after use, they should never be set on the table after they are unwrapped.

Sterile technique procedure

- Each lab group will spread one type of bacteria on several different types of nutrient media plates.
- When you receive your plates, turn them over so the nutrient agar is on the top side of the Petri dish, but do not open the plates!
- Lay out all the things you will need for the procedure

Sterile technique procedure

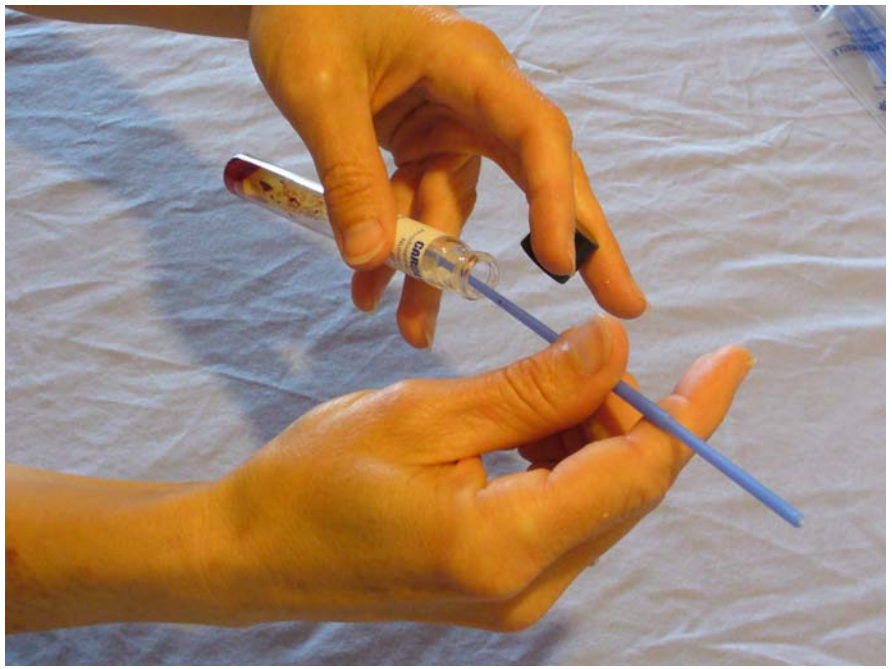
- With a permanent marker write the name of the nutrient media, the strain of bacteria you will be using and your lab group number/name on each plate.
- Read through the next few slides to get an idea of the procedure. Watch your teacher demonstrate the technique and as questions as needed.

Sterile technique procedure

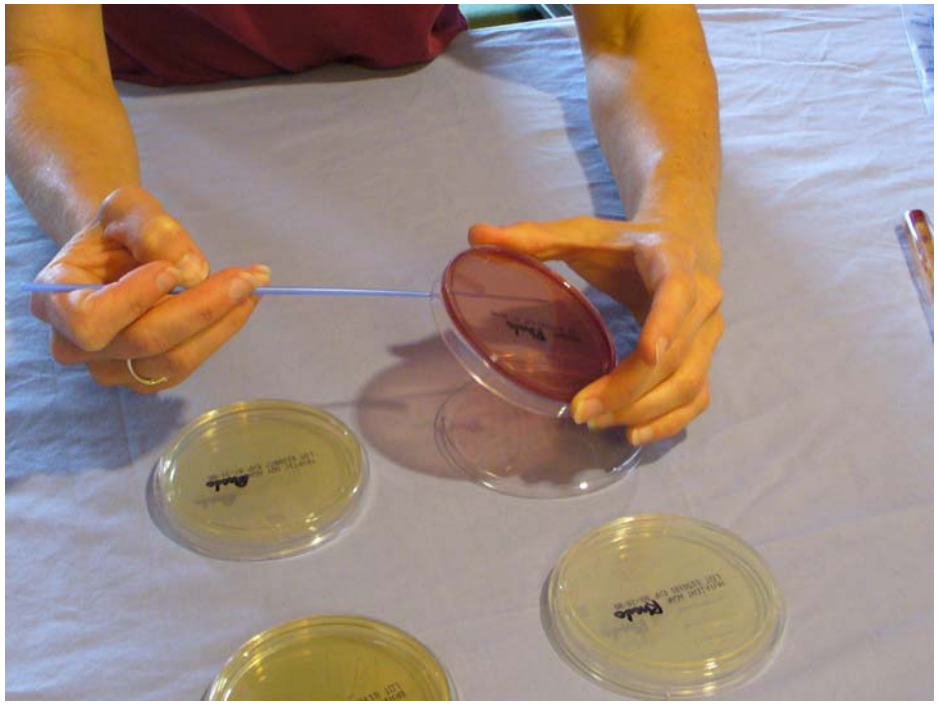
- If there are extra media plates, practice an inoculation without actually using any bacteria until your lab partner tells you it has been performed correctly with the minimal possibility of contamination

Sterile technique procedure

- To inoculate a plate with a particular strain of bacteria, open the tube of source bacteria and slide the rounded end of the inoculating loop around in the bacteria (do not set the cap down and recap the source tube immediately).

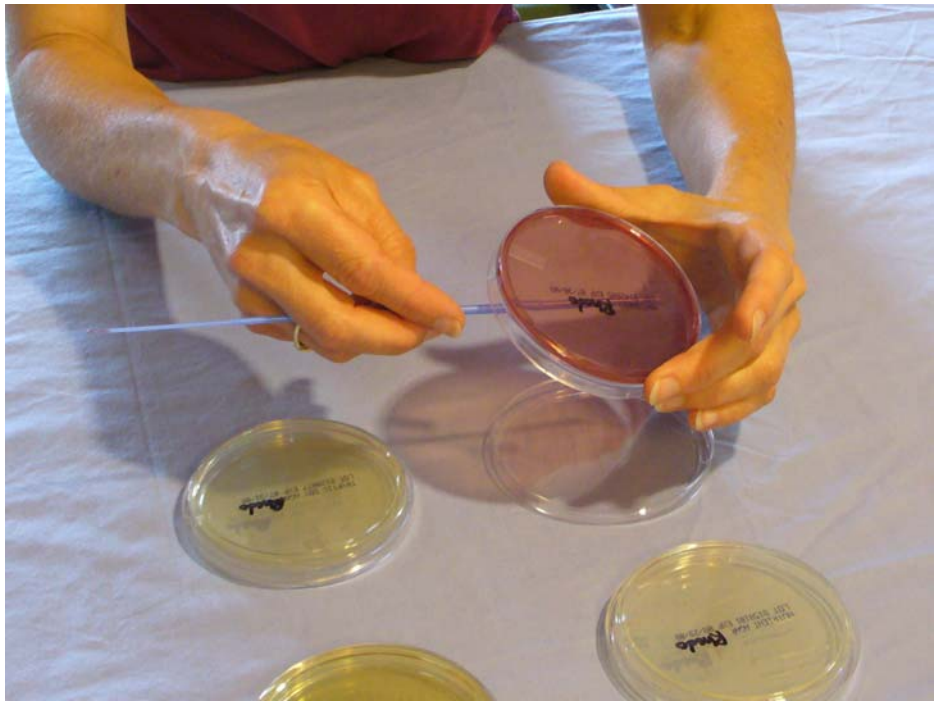


Sterile technique procedure



- Without touching the loop to any other surface, lift the media plate open as if it were a clam, barely opening its shell about 8-10 cm.
- Slide the inoculating loop in a zig-zag motion across one half of the agar media to evenly spread the bacteria.

Sterile technique procedure

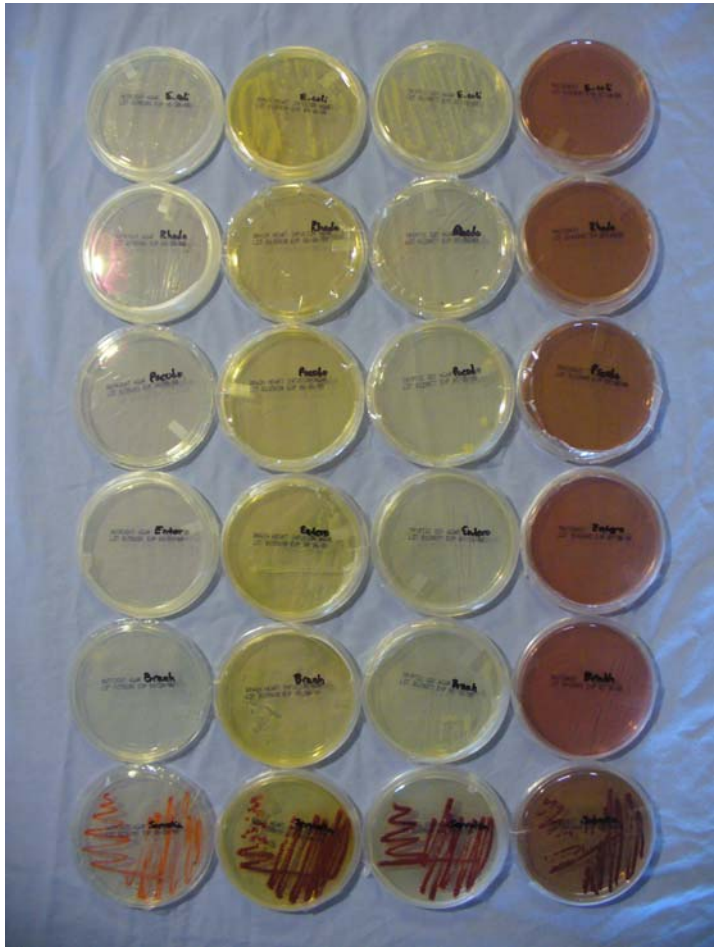


- Without touching either end of the loop to any other surface, flip the inoculating loop over to use the clean end to spread a single streak over into the other end of the media plate.

Sterile technique procedure

- Seal all media plates using Parafilm or tape as directed by your teacher.
- The media plates should never be reopened for any reason after they are inoculated.

Sterile technique procedure



- Place the media plates at room temperature out of direct sunlight where they can grow in a controlled environment.
- Wash your lab area and hands with soap.

Interpreting the results

- The bacteria will need time to grow on the media, when you see circular spots that indicates that bacterial colonies are forming signifying the bacteria strain can use the nutrients in that particular environment readily.

Interpreting the results

- Yellow shiny colonies or black webs of growth indicate contamination of the media plate with fungi – this is not a sign of growth by the bacteria that were spread on the media plate.

Reflection questions

- What is the value of finding out whether a bacteria strain prospers easily or is more finicky on different media?
- Which bacteria would you choose if you wanted a strain that could grow in an environment that has a fluctuation of available nutrients?
- Which bacteria would you choose if you were cleaning up an area that did not have a source of oxygen?

Activity: Oil-consuming Bacteria

- Some bacteria can consume oil as a source of food because oil is a chain of carbon atoms.
- How could these bacteria be used for environmental decontamination?

Oil-consuming bacteria procedure

- During this activity you will place a small amount of oil-consuming bacteria in a flask with nutrients and water.
- You will provide oxygen and a constant temperature to allow the bacteria to grow and multiply for a few days.

Oil-consuming bacteria procedure

- You will then add different types of oil to each flask to observe differences in the bacteria's ability to consume various types of hydrocarbon chains.

Oil-consuming bacteria procedure

- The oil-consuming bacteria will need 1-2 weeks to grow and prosper.
- While they are growing, record any changes you notice in the liquid in the flask – bubbles, foam, changes in the oil layer on the top of the flask, color or opacity of the nutrient media, etc.

Reflection questions

- What are the qualities of a well designed lab?
- How would you improve the procedure of this lab if you were to do it again?
- How can you tell if the bacteria were consuming the oil in the container?
- How do you think you could encourage faster consumption of the oil in the flask?
- If you had discovered this strain of bacteria, how could you best use these bacteria to help clean up environmental contamination?

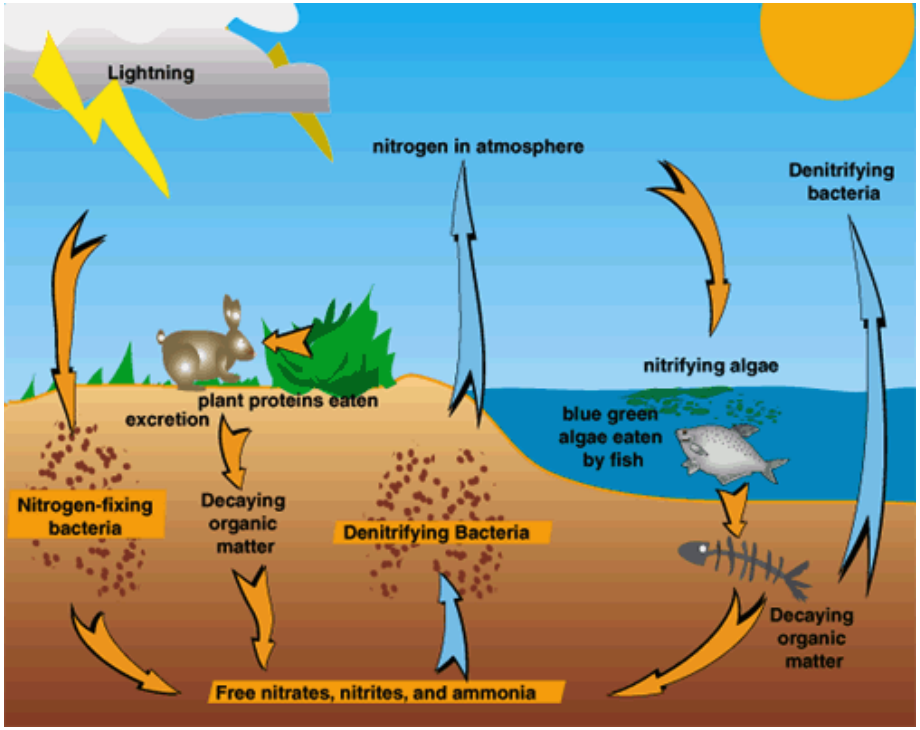
Part C: Waste water field trip

- In Part C you will see an example of plants, bacteria, algae, worms, snails and other invertebrates being used for bioremediation
- Microorganisms can be fed the nutrients in sewage as a method of purifying water

Human waste contains the nutrients we eat

- Sewage contains high levels of N, P, suspended solids and dissolved suspended solids, a high biological oxygen demand (BOD), and a low level of dissolved oxygen (DO)

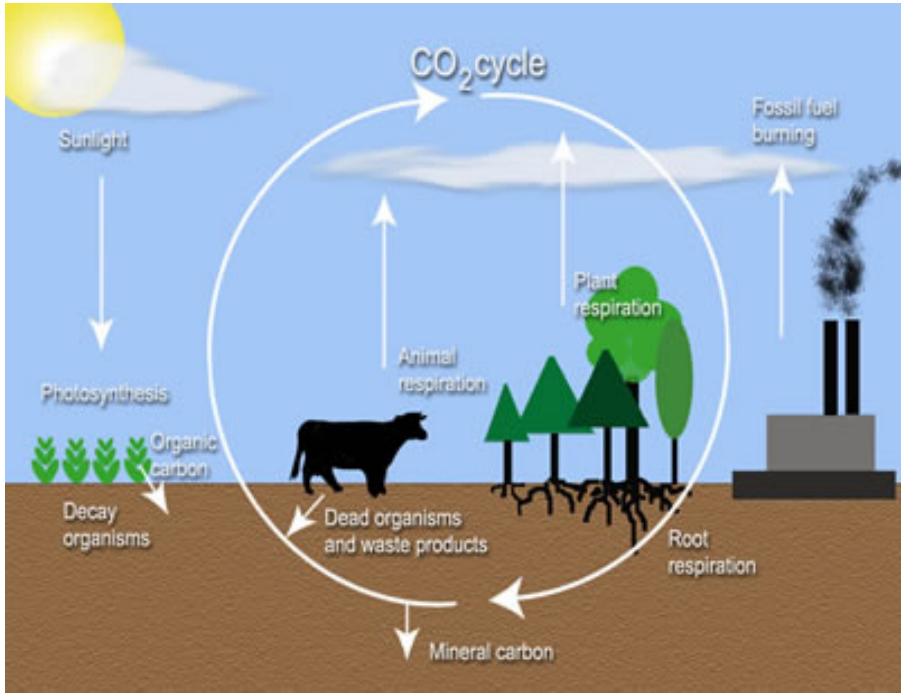
Biogeochemical nutrient cycles



- All nutrients (such as nitrogen, carbon, oxygen, phosphorus or sulfur) cycle through the abiotic and biotic environment
- These elements are constantly moving in and out of living and non-living things

Biogeochemical nutrient cycles

- The cycles interact as atoms bond to form new molecules with other elements in a different form
- Some forms of these elements can be a food source for one type of organism even if they are toxic to another



Sewage treatment encourages natural processes

- Suspended solids are removed by filters but the dissolved solids (aka nutrients) remain
- Imagine a glass of sweetened tea – the ice represents the suspended solids which are easy to remove
- The sugar represents the dissolved solids which are very difficult to remove

Sewage treatment encourages natural processes

- Bacteria, algae and other microorganisms are encouraged to grow by aerating the sewage and giving these organisms a substrate to grow upon
- The bacteria and other microorganisms consume the dissolved solids as a food source to gain N, C, S, P and other necessary components of their diet

Sewage treatment encourages natural processes

- In some treatment facilities, plants are used as a leach field to decompose any residual nutrients still in the water when the process is complete
- In other treatment facilities, the water may be sterilized using filters or chlorine

Activity: Field Trip to Sewage Treatment Plant

- You will visit a sewage treatment plant in your area to learn how large volumes of water contaminated with human waste can be cleaned for release into the environment.
- You will learn about the stages of the sewage treatment process.
- You will discover the essential role living organisms play in decontaminating sewage.

Reflection questions

- Explain how a sewage treatment facility uses bioremediation.
- Why does a scientist at a sewage treatment facility want to keep the microorganisms alive?
- Draw a graph of how the concentration of dissolved solids would correlate with the population of microorganisms.
- Predict what would happen if the incoming sewage pipes brought in water that was contaminated with a high level of chlorine?

What applications can you imagine for bioremediation?

- What are the problems that still need to be addressed?
- What solutions still need to be perfected?
- Where do you see this field going in the future?
- What can we do about the production of toxins and stopping contamination at the source?