Microbial decomposition of mineral oil

Students will learn how to perform cultural detection of mineral oil degrading microorganisms in soil.

Biology | Microbiology & genetics | Basics of microbiology

Difficulty level	Group size	Preparation time	Execution time
medium	1	20 minutes	30 minutes

This content can also be found online at:

http://localhost:1337/c/61dbf93aa2474d0003e08a99

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General information

Application

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Soil contamination by mineral oil

Some of the microorganisms, which are present in soil and water, are capable of utilizing petroleum components as carbon and energy sources. They break down these compounds to carbon dioxide and water, decontaminating the soil.

Mycobacteria, corynebacteria and proactinomycetes are particularly capable of degrading mineral oil.

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Other information (1/3)

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Prior knowledge

Students should already be familiar with the theoretical process of microbial degradation of mineral oil.

Scientific principle

Some microorganisms break down petroleum components into carbon dioxide and water, converting the mineral oil that pollutes the soil into harmless substances.

Other information (2/3)

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Learning objective

Students will learn how to perform cultural detection of mineral oil degrading microorganisms in soil.

Tasks

Students check for the presence of mineral oil-degrading microorganisms in Petri dishes.

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Other information (3/3)

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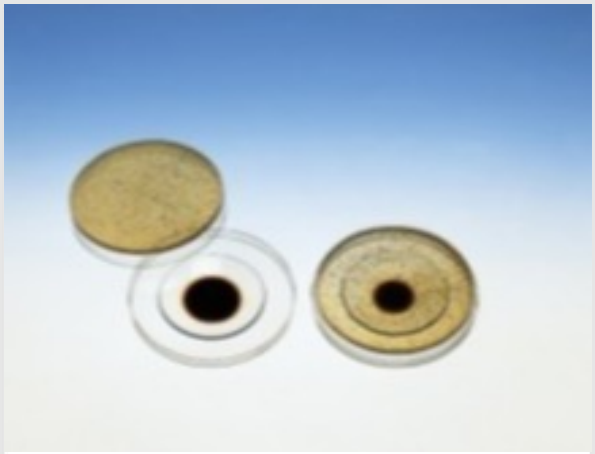
Observations and results

- Within 3-4 weeks, small translucent bacterial colonies develop on the culture medium. However, since this does not contain any carbon source, only those species that can utilize the mineral oil evaporating from the watch glass dish as a carbon source were able to develop.
- Mineral oil that enters the soil is broken down by these microorganisms and thus removed. However, this is only possible if this natural self-cleaning system is not overtaxed or even destroyed by excessive pollution of the soil with mineral oil.



Petri dishes with nutrient medium, soil and mineral oil

Safety instructions

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- The general instructions for safe experimentation in science education apply to this experiment.

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Theory

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Oil disasters are always a kind of nature disaster for the sensitive ecological systems in which they occur. The quantities of crude oil that are released into nature leave behind permanent damage and can only be removed with the utmost effort.

Smaller contaminants in soils and waters, on the other hand, can be degraded by the microorganisms present, provided the soils and waters are intact.

Mycobacteria, corynebacteria and proactinomycetes, which break down mineral oils to carbon dioxide and water, are particularly capable of this.

Mineral oil that enters the soil is broken down by these microorganisms and thus removed. However, this is only possible if this natural self-cleaning system is not overtaxed or even destroyed by excessive pollution of the soil with mineral oil.

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Equipment

Position	Material	Item No.	Quantity
1	Autoclave with insert	04431-93	1
2	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
3	Test tube rack for 12 tubes, holes d= 22 mm, wood	37686-10	1
4	Test tube, 160 x 16 mm, 100 pcs	37656-10	1
5	Sterile stoppers f. id 15mm, 250	39266-00	1
6	Spoon, with spatula end, 180 mm, plastic	38833-00	1
7	Graduated cylinder, Borosilicate, 250 ml	36630-00	1
8	Glass rod,boro 3.3,l=300mm, d=7mm	40485-05	1
9	Beaker, Borosilicate, tall form, 600 ml	46029-00	1
10	Tripod,ring d=140 mm, h=240 mm	33302-00	1
11	Wire gauze with ceramic, 160 x 160 mm	33287-01	1
12	Bunsen burner /DIN/,nat.g.,w.cock	32168-05	1
13	Safety gas tubing, DVGW, sold by metre	39281-10	1
14	Petri dish, d 100 mm	64705-00	2
15	Watch glass, dia.60 mm	34570-00	2
16	Agar-agar, powdered 100 g	31083-10	1
17	Ammonium chloride 250 g	30024-25	1
18	Potas.dihydrogen phosphate,100 g	30261-10	1
19	Magnesium sulphate 500 g	30136-50	1
20	Water, distilled 5 l	31246-81	1

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Set-up and procedure

Set-up (1/3)

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For the cultural detection of mineral oil degrading microorganisms in soil, tubes with nutrient agar for mycobacteria are required. They are to be prepared as follows before starting the actual experiment:

- Fill a beaker with distilled water and add
  - 0.05% ammonium chloride,
  - 0.05% potassium dihydrogen phosphate
  - 0.05% magnesium sulfate

is added. Add 2 % agar and boil until the agar is completely dissolved. Since it foams very strongly during boiling and consequently boils over easily, the beaker must be large enough to hold three to four times the amount of culture medium prepared. Therefore, as soon as the solution boils, continue heating only over a low flame and stir frequently with a glass rod.

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Set-up (2/3)

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- The finished culture medium is poured into test tubes (well half full).
- Close each test tube with a sterile stopper and sterilize in the steamer for half an hour from the start of boiling.
- The sterilization is repeated in the same way on the following two days.
- This fractional sterilization is necessary because heating once to the boiling temperature of the water only kills the vegetative stages of the microorganisms, but not the bacterial spores.
- The surviving spores germinate in the cooled nutrient agar to form vegetative cells, which are killed by the second sterilization.
- For safety, sterilize again on the third day.

Set-up (3/3)

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- The test tubes with the nutrient agar are brought into the steam pot in several in one container, the easiest way is in an empty tin can.
- The sterile stoppers of the test tubes must be covered with a piece of parchment paper so that condensation dripping from the lid of the steam cooker cannot soak them.
- If a pressure pot or autoclave is available, a single sterilization of 20 minutes after reaching a positive pressure of 1.2 atmospheres is sufficient.

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Procedure

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- The contents of two tubes containing nutrient agar for mycobacteria are liquefied by boiling in a water bath. A beaker (600 ml) filled approximately two-thirds with water serves as the water bath.
- The liquefied culture medium is poured out in each case into a Petri dish (diameter 100 mm), which need not be sterile.
- Dust some air-dry garden or field soil onto the solidified culture medium by rubbing it between the fingers. The Petri dishes are closed with the lid and turned around so that the bottom is facing upwards.
- Place a watch glass dish (diameter 60 mm) with some mineral oil (gasoline, fuel oil or kerosene) in each lid, and leave the dishes at room temperature.
- Within 3-4 weeks, small translucent bacterial colonies develop on the culture medium. However, since this does not contain any carbon source, only those species that can utilize the mineral oil evaporating from the watch glass dish as a carbon source were able to develop.

Report

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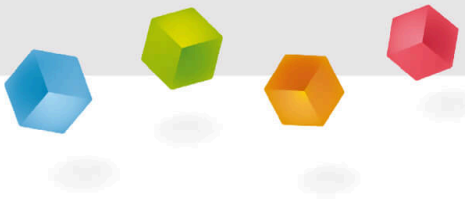

Report

Task 1

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What do the microorganisms present in the soil and water break down the mineral oil components into?

- ○ The microorganisms break down the mineral oil components to carbon dioxide and water and use them as an energy source.
- ○ The microorganisms break down the mineral oil components to nitrogen and water and use them as an energy source.
- ○ The microorganisms break down the mineral oil components to oxygen and water and use them as an energy source.

Check

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Task 2

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If microorganisms are able to break down mineral oil, why is there an outcry around the world every time an oil spill happens?

- ○ Because with oil spills, the attention of many is drawn to nature. Actually, oil is never a problem in nature.
- ○ Because especially in seawater no microorganisms occurred, because the salt content there is much too high.
- ○ Degradation is only possible if the contamination is not too great, as the microorganisms are then overtaxed or even destroyed.

Check

Task 3

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Who is qualified to extract mineral oil?

- ○ Mycobacteria, corynebacteria and proactinomycetes are particularly capable of degrading mineral oil.
- ○ Staphylococci, enterobacteria and pseudomonads are particularly capable of degrading mineral oil.
- ○ Protozoa, helminths and hepatitis viruses are particularly capable of degrading mineral oil.

Check

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