Microbial decomposition of mineral oil (Item No.: P4100900)

Curricular Relevance

Area of Expertise: Biology

Education Level: University

Topic: Microbiology

Subtopic: Microbiology

Experiment: Microbial decomposition of mineral oil

Difficulty	Preparation Time	Execution Time	Recommended Group Size
Intermediate	20 Minutes	30 Minutes	1 Student

Additional Requirements:

Experiment Variations:

Keywords:

Mineral oil, Contamination, Mycobacteria, Microorganisms

Overview

Application and principle

Soil contamination by mineral oil

Some microorganisms living in soil and water are capable of utilizing mineral oil compounds as carbon and energy sources. They break down these compounds to produce carbon dioxide and water, thereby decomposing mineral oil. Particularly mycobacteria, corynebacteria and proactomycetes are able to decompose mineral oil.

Student's Sheet

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Equipment

Position No.	Material	Order No.	Quantity
1	Autoclave with insert	04431-93	1
2	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
3	Bunsen burner /DIN/,nat.g.,w.cock	32168-05	1
4	Agar-agar, powdered 100 g	31083-10	1
5	Sterile stoppers f. id 15mm, 250	39266-00	1
6	Test tube rack for 12 tubes, holes d= 22 mm, wood	37686-10	1
7	Test tube, 160 x 16 mm, 100 pcs	37656-10	1
8	Tripod,ring d=140 mm, h=240 mm	33302-00	1
9	Magnesium sulphate 500 g	30136-50	1
10	Ammonium chloride 250 g	30024-25	1
11	Water, distilled 5 l	31246-81	1
12	Graduated cylinder 250 ml	36630-00	1
13	Safety gas tubing, DVGW, sold by metre	39281-10	1
14	Potas.dihydrogen phosphate,100 g	30261-10	1
15	Glass beaker DURAN®, tall, 600 ml	36006-00	1
16	Wire gauze with ceramic, 160 x 160 mm	33287-01	1
17	Spoon,w.spatula end,18 cm,plastic	38833-00	1
18	Petri dish, d 100 mm	64705-00	2
19	Glass rod,boro 3.3,l=300mm, d=7mm	40485-05	1
20	Watch glass, dia.60 mm	34570-00	2

Task

Verify the presence of mineral oil-degrading microorganism in soil test tubes.

Set-up and procedure

Set-up

To verify the presence of mineral oil-degrading microorganism in soil test tubes filled with nutrient agar for mycobacteria are required. Make them up prior to performing the experiment:

Add the following substances to distilled water in a beaker:

Ammonium chloride: 0.05%
Potassium dihydrogen phosphate: 0.05%
Magnesium sulphate: 0.05%

as well as 2% agar-agar. Boil until agar-agar is completely dissolved. Since agar-agar generates plenty of foam during boiling, the beaker must have a volume which is three to four times that of the required amount of medium. Therefore reduce the flame once the solution has come to a boil and stir with a glas rod.

Fill the medium in test tubes to half their volume. Close each test tube with a sterile stopper and sterilize in the autoclave for half an hour (make sure you start counting only once the temperature has reached boiling point). In the next two days sterilization is repeated in the same way. This fractionated sterilization is required because after one-time heating only the vegetative forms of the microorganisms are killed, however not their spores. The surviving spores germinate in the cooled down nutrient agar forming new vegetative cells, which are then killed in the second sterilization step. For reasons of safety sterilization is repeated on the third day.

Several test tubes with the nutrient agar are put together in an insert into the autoclave. This is best done by using an empty tin can. The sterile stoppers of the test tubes must be completely covered with a piece of wrapping paper so that they cannot become wet due to condensation water dripping down from the autoclave top.

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Procedure

The content of two test tubes with nutrient agar for mycobacteria is liquefied by boiling in a water bath, which is made up of a 600 ml beaker filled with water to two thirds of the beaker's total volume. The liquefied nutrient agar is poured into one petri dish (10 cm diameter) for each test tube. It is not necessary that the petri dishes are sterile.

Put some air-dried garden soil onto the solidified nutrient agar. Make sure not to use coarse lumps of soil but rather fine soil fractions.

Fig. 1: Petri dishes with nutrient medium, soil and mineral oil

Close the petri dishes with their lids and turn them around so that the bottom is up. Put a 60 mm watch glass which contains some mineral oil into each petri dish cover and let the petri dishes stand at room temperature.

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Observations and results

Results and evaluation

Within three to four weeks small translucent bacterium colonies show on the nutrient agar. Since the nutrient agar does not contain any carbon source, only those species can develop which can utilize the evaporating mineral oil as the carbon source.

Mineral oil which has contaminated soil is degraded by these microorganisms and eliminated. However, this process is only possible if the natural self-purification system is not overburdened or even destroyed by excessive mineral oil contamination of the soil.

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