
Lab5: Incubation of bacteria

Temperature is one of the most important factors that influence the enzymatic activity of microorganisms. So the enzymes are controlling microbial growth and they have minimum, optimum and maximum temperature.

At the optimum temperature the enzymatic reaction progress at a maximum speed, but below the minimum or above the maximum temperature the enzymes become inactive.

Microorganisms are dividing into three groups based on their preferable temperature ranges:

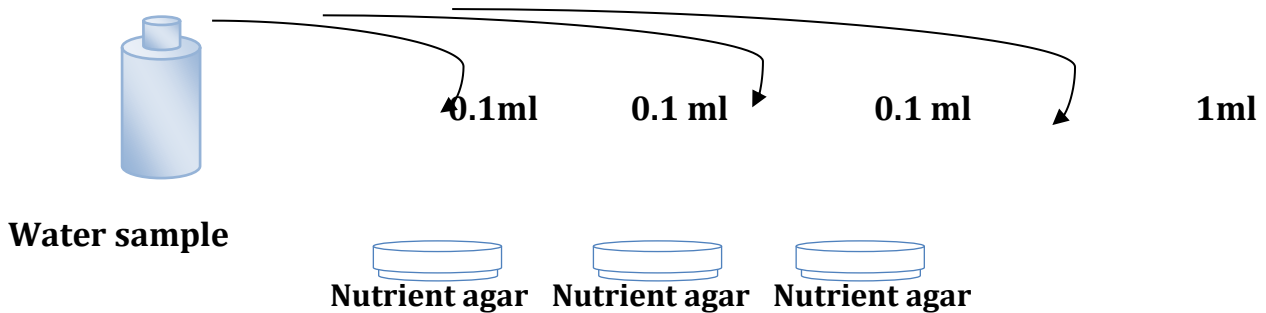
1. **Psychrophilic:** cold-loving microorganisms (**0-25°C**).
 - a. Obligate psychrophilic: microorganisms that **cannot grow** above 25°C.
 - b. Facultative psychrophilic: microorganisms that **can grow** above 25°C.
2. **Mesophiles:** moderate temperature-loving microorganisms (**10-47°C**).
3. **Thermophiles:** hot-loving microorganisms (**above 50°C**).
 - a. Obligate thermophiles: microorganisms that grow above 50°C.
 - b. Facultative thermophiles: microorganisms that grow below 50°C.

Note: the *disease causing and spoilage microorganisms* are mostly Mesophiles.

The advantages of incubators are that they may be set at a range of temperatures and reduce the possibility of cultures being interfered with or accidentally discarded. However, many cultures suitable for use in schools will grow at room temperature in the interval between lessons and can be incubated satisfactorily in a cupboard.

The temperature of an incubator varies from the set temperature, oscillating by several degrees in the course of use.

Water baths are used when accurately controlled temperatures Are required, e.g. for enzyme reactions and growth-temperature Relationships, when temperature control of incubators is not sufficiently precise. They should be used with distilled or deionised water to prevent corrosion and emptied and dried for storage.

Procedure:

Add about 12-15ml of Nutrient agar to each plate



**Invert and incubate the plates at different temperatures
(4°C, 37°C and 50°C) for 24-48hr.**



Record your results in a regulated laboratory report.

Figure 1: Effect of temperature on microbial growth

✓ Notes:

- **labelling the plate with all enough information as in figure**
- **The lid and base of an agar plate should be taped together with short strips of adhesive tape as a protection from accidental (or unauthorised!) opening during incubation.**
- **Agar plates must be incubated with the medium-containing half (base) of the Petri dish uppermost otherwise condensation will occur on the lid and drip onto the culture. This might cause colonies to spread into each other and risk the spillage of the contaminated liquid.**
- **Overlong incubation of mould cultures will result in massive formation of spores which readily escape, particularly from Petri dishes, and may cause contamination problems in the laboratory and be a health hazard. This can occur in an incubator, at room temperature and even in a refrigerator.**



Figure 2: labelling the culturing plate before incubation

- **Examination of Culture Growth**

1. When you have finished making the culture transfers as directed, take a few minutes to look closely at the grown cultures with which you have been working. In the Results section of this exercise, there are blank forms in which you can record information as to the appearance of these cultures, specifically: size of colonies (in mm), color, density (translucent? opaque?), consistency (creamy? dry? flaky?), surface texture (smooth? rough?), and shape of colony (margin even or serrated? flat? heaped?).
2. When the cultures you have made have grown out, record their appearance in broth or on slants, using the blank form in the Results section.

- **A. Examination of growth in broth growth**

In liquid media, some bacteria grow diffusely, producing uniform clouding, whereas others look very granular. Layering of growth at the top, centre, or bottom of a broth tube reveals something of the organisms' oxygen requirements. Sometimes colonial aggregates are formed and the bacterial growth appears as small puff balls floating in the broth. Observation of such features can also be helpful in recognizing types of organisms. Examinations of growth in broth growth of bacteria have the following characterize (**Sediment, pellicle, Ring, Turbid, and Flocculent**)

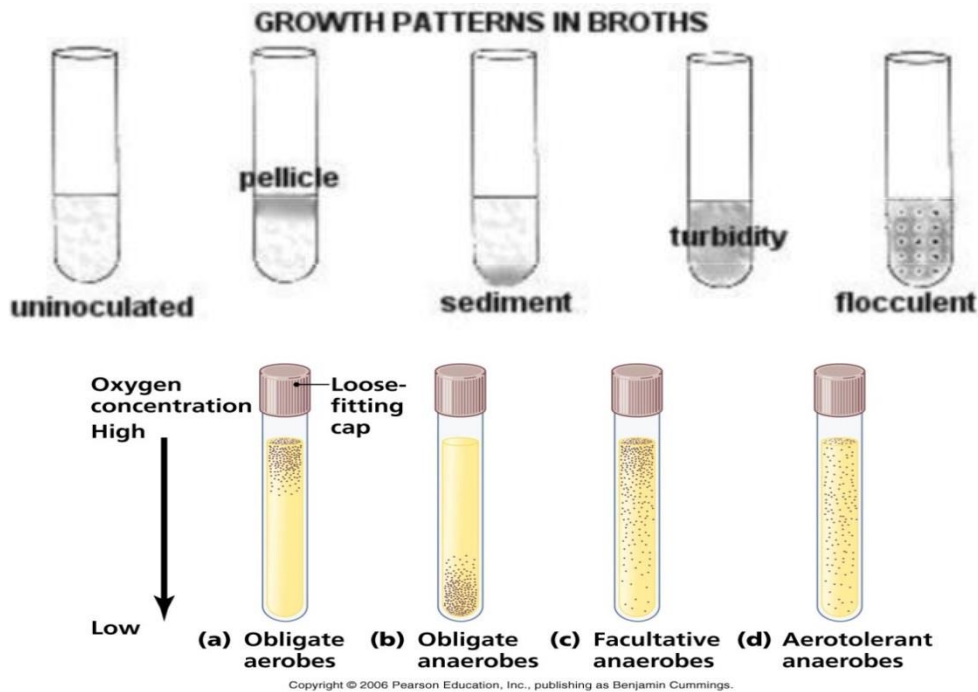


Figure 3: Shapes of bacteria on broth after incubation.

B. Examination of growth on agar

1. slant

The growths of bacteria on agar slant have the following characterize (Fusiform, Beccid, Echinulate, Effuse, Arborescent, and Rhizoid)

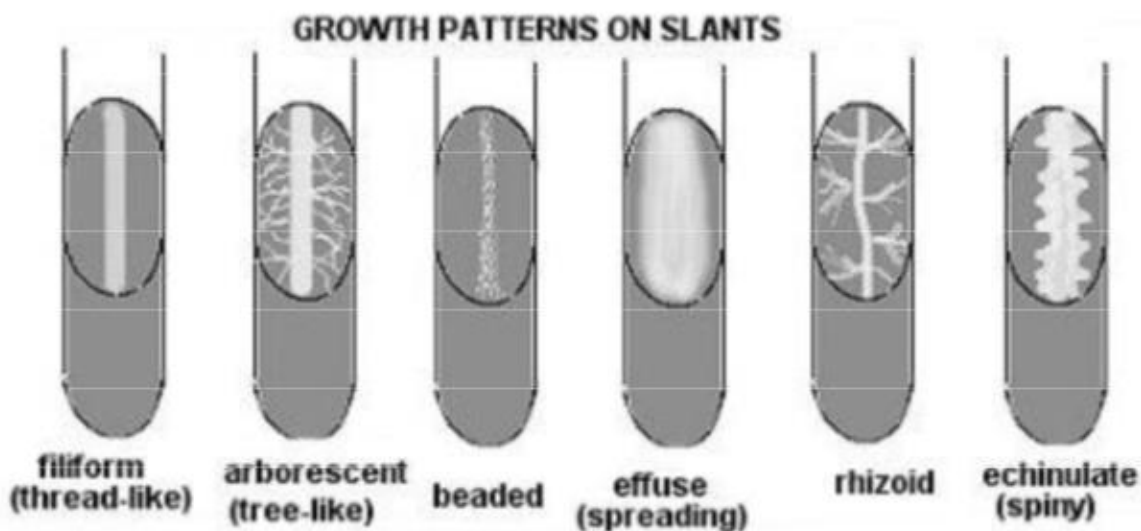


Figure 4: growth bacteria on slant

Bacterial Cultures on an Agar Slant

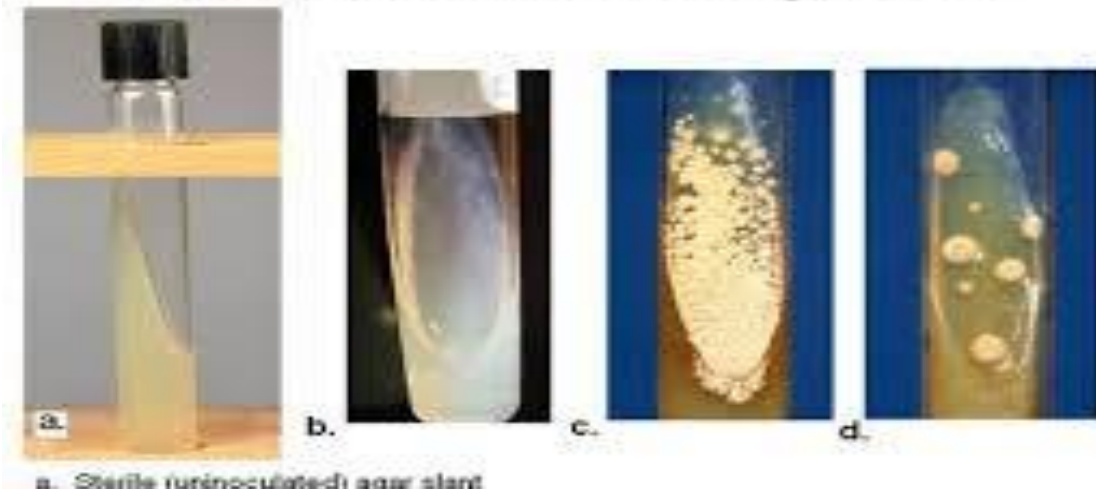
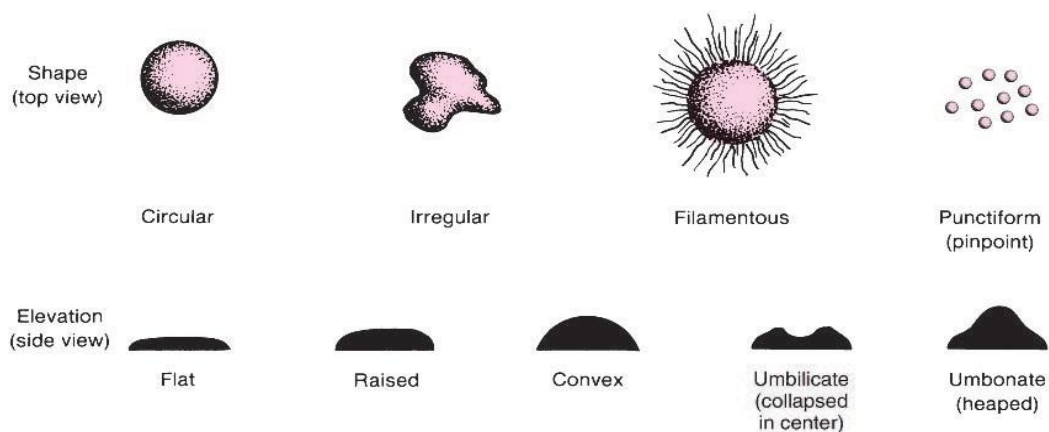


Figure 5: shapes of bacteria on agar slant.

2. plate

By characterizing of shape, margin, elevation, size, appearance. Pigmentation, and optical characterizes. The appearance of colonial growth on agar media can be very distinctive for individual species. Observation of the noticeable, gross features of colonies, that is, of their colonial morphology, is therefore very important. The colour, density, consistency, surface texture, shape, and size of colonies all should be observed, for these features can provide clues as to the identity of an organism, although final identification cannot be made by morphology alone.



(a) Some colonial characteristics on agar media.*

Figure 6: shapes of bacteria on agar plate