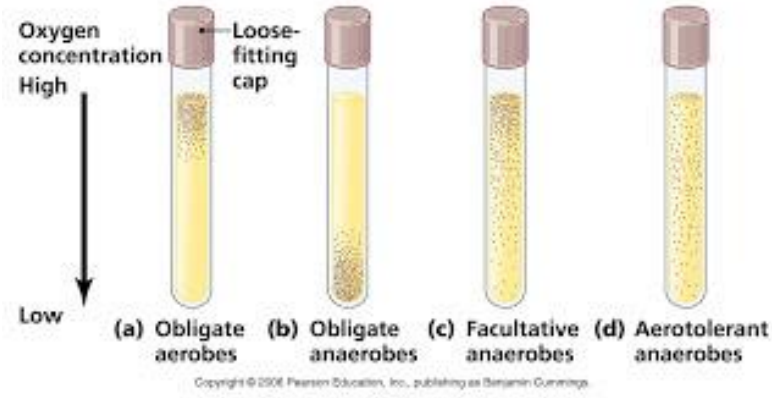
Cultivation of Anaerobic Bacteria

Main Principle: reduce the O_2 content of culture medium and remove any oxygen already present inside the system or in the medium .

Oxygen is ubiquitous in the air so special methods are needed to culture anaerobic microorganisms.

A number of procedure are available for **reducing the O2 content** of cultures; some simple but suitable mainly for less sensitive organisms, others more complex but necessary for growth of strict anaerobes.

- Bottles or tubes filled completely to the top with culture medium and provided with tightly fitting stopper. Suitable for organisms not too sensitive to small amounts of oxygen.
- Addition of a reducing agent that reacts with oxygen and reduces it to water e.g., Thioglycolate in thioglycolate broth. After thioglycolate reacts with oxygen throughout the tube, oxygen can penetrate only near the top of the tube where the medium contacts air.
    - Obligate aerobes grow only at the top of such tubes.
    - Facultative organisms grow throughout the tube but best near the top.
    - Microaerophiles grow near the top but not right at the top.
    - Anaerobes grow only near the bottom of the tube, where oxygen cannot penetrate.



A redox indicator dye called resazurin is added to the medium because the dye changes color in the presence of oxygen and thereby indicates the degree of penetration of oxygen into the medium.

Strict anaerobes, such as methanogenic bacteria can be killed by even a brief exposure to O_2. In these cases, a culture medium is first boiled to render it oxygen free, and then a reducing agent such as H_2S is added and the mixture is sealed under an oxygen- free gas. All manipulations are carried out under a tiny jet of oxygen free hydrogen or nitrogen gas that is directed into the culture vessel when it is open, thus driving out any O_2that might enter. For extensive research on anaerobes, special boxes fitted with gloves, called anaerobic glove boxes, permit work with open cultures in completely anoxic atmospheres.

Stringent anaerobes can be grown only by taking special precautions to exclude all atmospheric oxygen from the medium. Such an environment can be established by using one of the following methods:

1. **Pre-reduced media**

   During preparation, the culture medium is **boiled** for several minutes to drive off most of the dissolved oxygen. A reducing agent e.g., cysteine, is added to further lower the oxygen content. Oxygen free N_2 is bubbled through the medium to keep it anaerobic. The medium is then dispensed

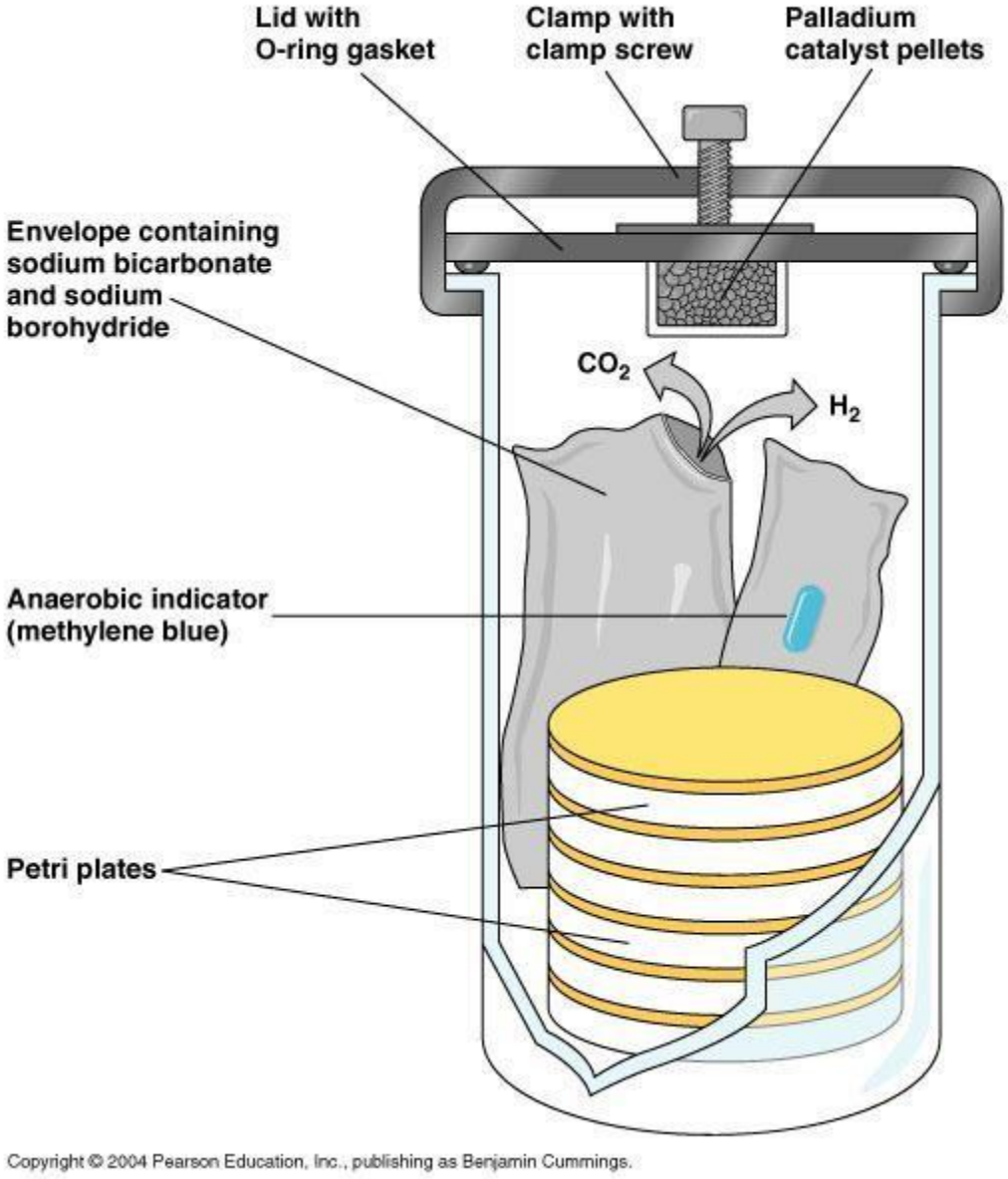
into tubes which are being flushed with oxygen – free nitrogen, stoppered tightly, and**sterilized by autoclaving**. Such tubes are continuously flushed with oxygen free CO_2 by means of a cannula, restoppered, and incubated.

2. **Anaerobic Chambers**

Anaerobic Chamber

This refers to a plastic anaerobic glove box that contains an atmosphere of H_2, CO_2, and N_2. Culture media are placed within the chamber by means of an air lock which can be evacuated and refilled with N_2. Any oxygen in the media is slowly removed by reaction with hydrogren, forming water; this reaction is aided by a palladium catalyst. After being rendered oxygen free, the media are inoculated within the chamber (by means of the glove ports) and incubated (also within the chamber).

3. **Anaerobic Jar**



Anaerobic Jar: GasPak system

Anaerobic jar is a heavy- walled jar with a gas tight seal within which tubes, plates, or other containers to be incubated are placed along with H_2 and CO_2 generating system (GasPak system) . After the jar is sealed **oxygen** present in the atmosphere inside jar and dissolved in the culture medium, **is gradually used up** through reaction with the hydrogen in the presence of catalyst. The air in the jar is replaced with a mixture of H_2 and CO_2, thus leading to anoxic conditions.