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Suman Kumar Mekap
Asst. Professor
RIPS, Berhampur

BACTERIAL GROWTH CURVE

INTRODUCTION

Bacteria ‘s growth can be take place by binary fission and during that different events takes place.

Three type of growth curves:

1) Growth cycle

2)Synchronous growth

3) Bacterial growth in vivo

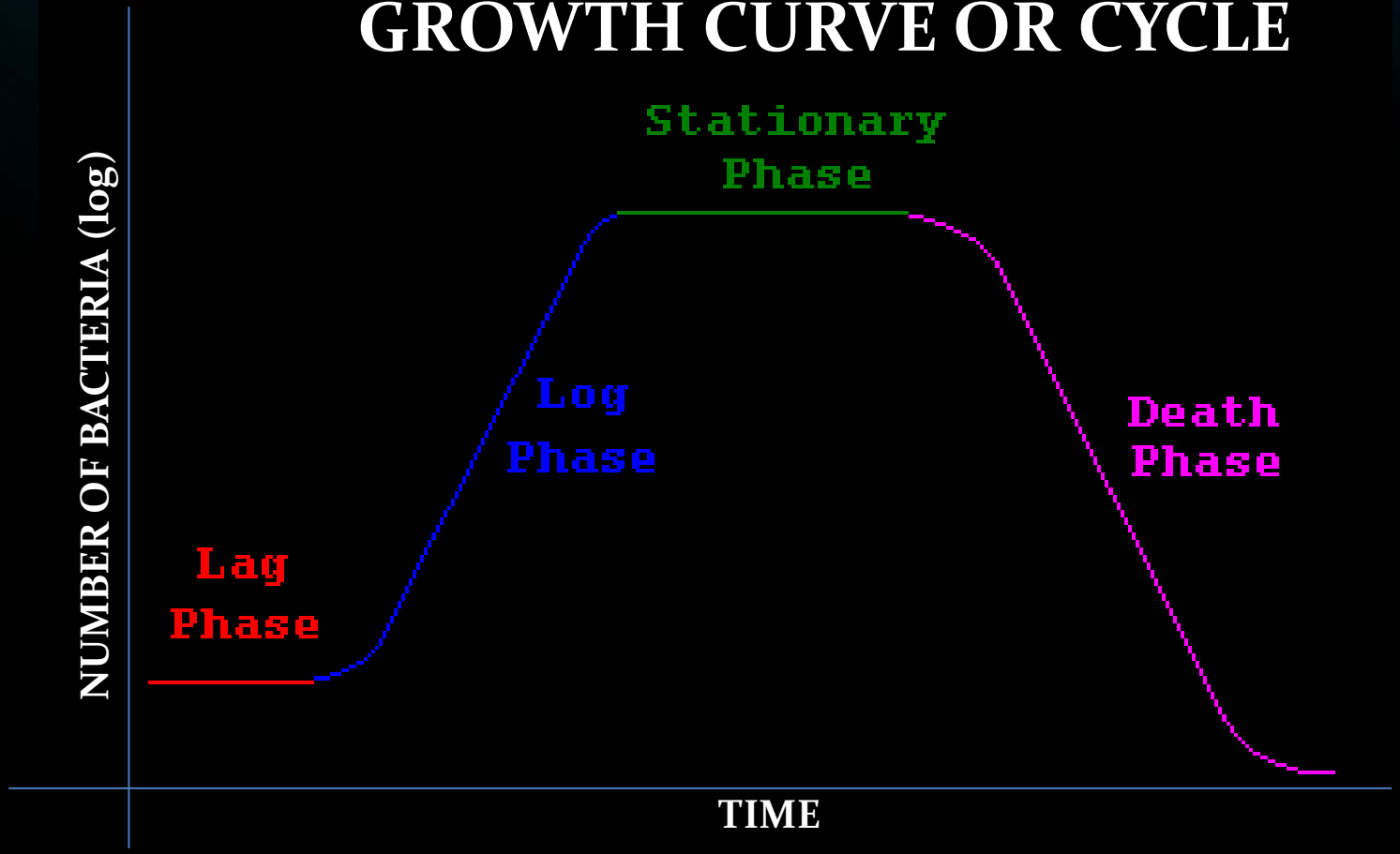
GROWTH CYCLE

Bacterial growth is regulated by nutritional environment. When suitable environment is there that time bacterium is incubated, its growth leads to increase in number of cells which allow definite course.

The growth curve has got four phases:

- Lag phase
- Log phase(logarithmic) or exponential phase
- Stationary phase
- Decline phase

GROWTH CURVE OR CYCLE



1. LAG PHASE (1-4 HOUR)

- bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide.
- During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
- Length of this phase depend on type of bacterial species, culture medium, and environmental factors.

2. LOG PHASE OR EXPONENTIAL (8 HOUR)

(sometimes called the logarithmic phase)

- It is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population.
- If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period.
- For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line.

- The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving.

- Under controlled conditions, cyanobacteria can double their population four times a day.

- Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

3. STATIONARY PHASE (FEW HOURS TO DAYS)

- The "stationary phase" is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids.

- An awkward but unfortunately widespread explanation is that the stationary phase results from a situation in which growth rate and death rate have the same values (newly formed cells per time = dying cells per time);but this is not logical, and it is better to forget this.

- Such an explanation would not be in accordance with the observed substrate depletion and also could never explain the rather “smooth,” horizontal linear part of the curve during the stationary phase.

- Death of cells as a function of time is rather unpredictable and very difficult to explain.

- Another not really logical explanation of the stationary phase is that there isn't anymore enough space for the cells However, under the microscope you will see that there is still plenty of water between the cells.

- Only in an agar colony with densely packed cells space is obviously limiting.

4. DECLINE (DEATH) PHASE (FEW HOURS TO DAYS)

- ✓ bacteria run out of nutrients and die although number of cells remain constant.

- ✓ The decline phase is brought by exhaustion of nutrients, accumulation of toxic products and autolytic enzymes.

- ✓ Sometimes a small numbers of survivors may persist for month even after death of majority of cells these few surviving cells probably grow at expense of nutrients released

SYNSYNCHRONOUS PHASE

- Bacteria grow nonsynchronously in ordinary culture medium, i.e at any moment cells are present in different stage of growth cycle.

- When all bacterial cells in culture medium divide simultaneously growth thus obtained is known as synchronous growth.

- Such growth is required for studding the sequence of event occurring in single cell like studies on DNA synthesis or susceptibility of cell to lethal agent.

BACTERIAL GROWTH *IN -VIVO*

- There exists a significant difference of bacterial growth in human body and artificial culture medium.

- They grow much faster *In-vitro* than *In-vivo*.

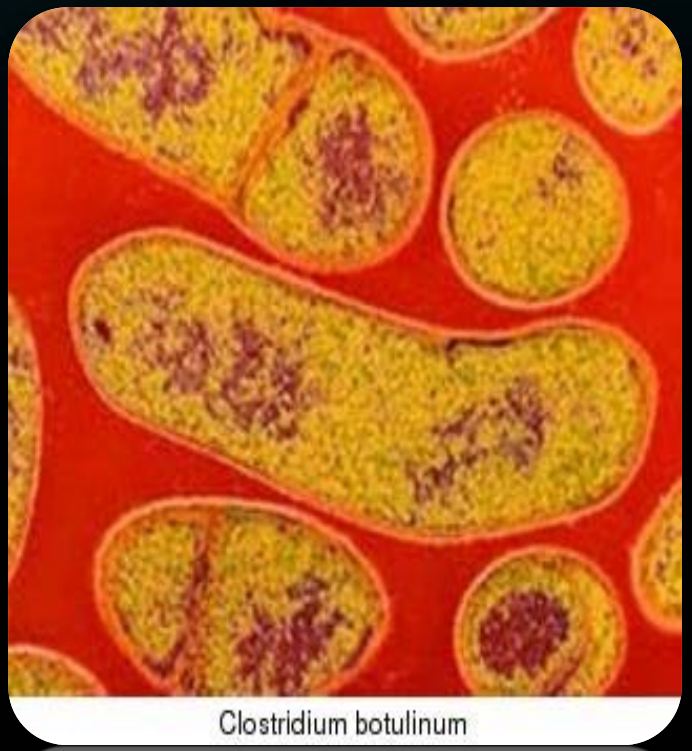
- Various factor in vivo include: nutritional status of body, generation time, defense mechanism, redox potential, hydrogen ion concentration , localisation of nutrients.

ANAEROBIC CULTURE METHODS

What Are Anaerobic Microorganisms

- Anaerobic microorganisms are widespread and very important

- Do not require oxygen for growth - often extremely toxic

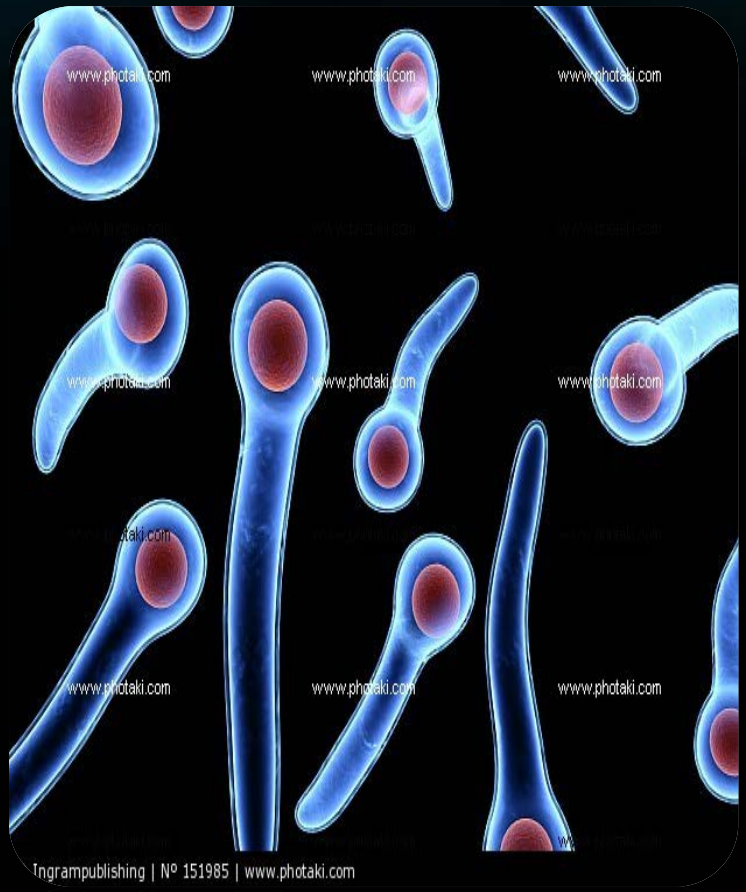


Defining Anaerobes

- **Facultative anaerobes** - can grow in the presence or absence of oxygen
- Obtain energy by both respiration and fermentation
- Some use nitrate (NO_3^-) or sulphate (SO_4^{2-}) as a terminal electron acceptor under anaerobic conditions

Strict Anaerobic Bacteria

- **Obligate (strict) anaerobes** - oxygen is toxic to these organisms, do not use oxygen as terminal electron acceptor.
- Archaea such as methanogens and Bacteria, e.g Clostridia, Bacteriodes etc. etc.



Culturing of anaerobes need special skills

- Culture of anaerobes is extremely difficult because
  - need to exclude oxygen,
  - slow growth and
  - complex growth requirements

- By molecular methods based on DNA analysis and direct microscopy have shown that anaerobic bacteria are diverse

Effects of Oxygen on Bacteria

- Obligate Aerobes—"strict aerobes"; oxygen required
- Facultative Anaerobes—both aerobic and anaerobic growth; greater growth in presence of oxygen
- Obligate Anaerobes—"strict anaerobes" only anaerobic growth; dies in presence of oxygen
- Aerotolerant Anaerobes—only anaerobic growth; but continues in presence of oxygen
- Microaerophiles—only aerobic growth; oxygen required in low concentration
- Capnophiles—microbes that grow better at high CO_2 concentrations; low oxygen high carbon dioxide conditions (resembles those found in the intestinal tract)

Anaerobic culture methods

1. Use of media containing reducing substances (Robertson Cooked Meat broth or Thioglycolate broth).

2. Culture away from O_2 (Deep agar tubes).

3. Chemical exclusion of O_2 (anaerobic Gas Pak system, Candle Jar).

4. Mechanical exclusion of O_2 (anaerobic incubator).

1- Use of media containing reducing substances.

A- Robertson Cooked Meat broth

- ✓ Composition: 5gm of cooked meat particles + nutrient broth.
- ✓ The reducing substances are haemin and glutathione in meat particles
- ✓ Uses: for anaerobic cultivation
- ✓ Sterilisation: Autoclave at 121°C for 30 min

- Robertson Cooked Meat Broth

B- Thioglycolate broth:

- Media for anaerobes supplemented with nutrients like hemin and vitamin K, 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings .

- Sterilize by autoclaving at 121°C for 15 minutes.

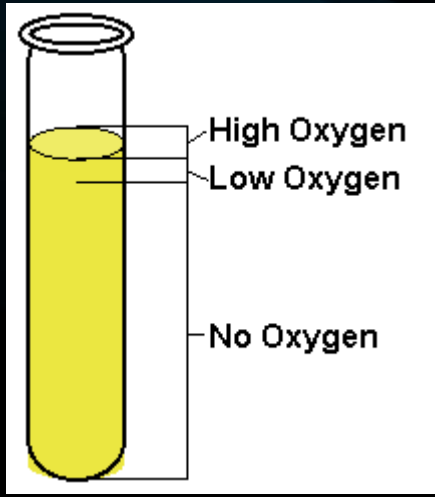
- Cool to 25°C and store in a cool dark place preferably below 25°C.

- Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Thioglycolate broth

Culture away from O2 (Deep agar tubes).

- Simple way to produce anaerobic condition
- The agar surface can be overlaid with oil to maintain the anaerobic condition.
- Sterilization of the media can be carried out in the autoclave at 121° C for 30 minutes.
- Inoculation is by deep stabbing.



O2 content of culture tube

Growth in deep agar

3- Chemical exclusion of O2 (anaerobic Gas Pak system)

Uses H2 to convert air O2 to H2O in the presence of a catalyst.

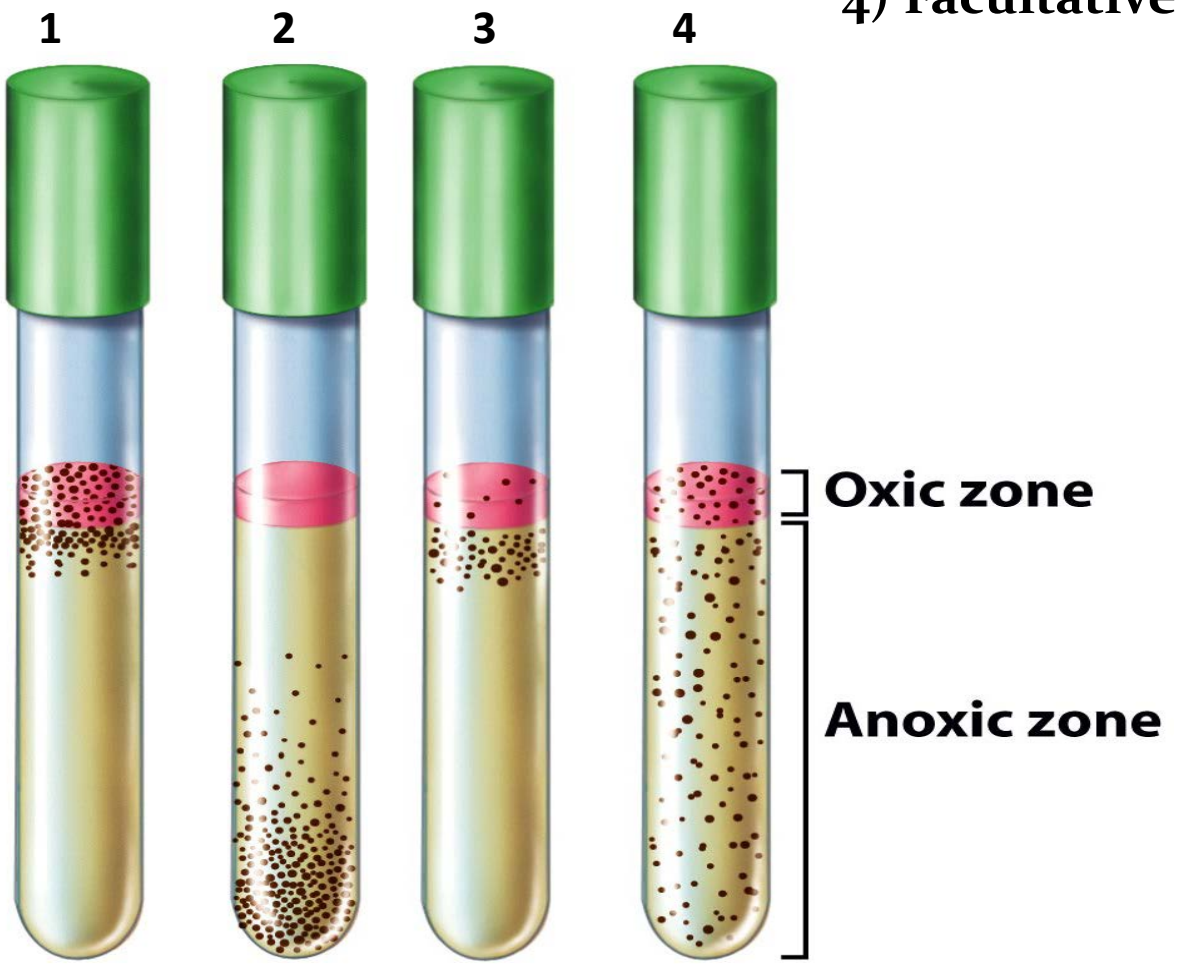
- The reaction formula is ($2H_2 + O_2 \longrightarrow 2H_2O$).
- The source of H2 is the Gas Packet commercially supplied.
- The catalyst is palladium contained in the lid of the jar.
- Anaerobic indicator strips included to monitor the anaerobic condition.

Procedure:

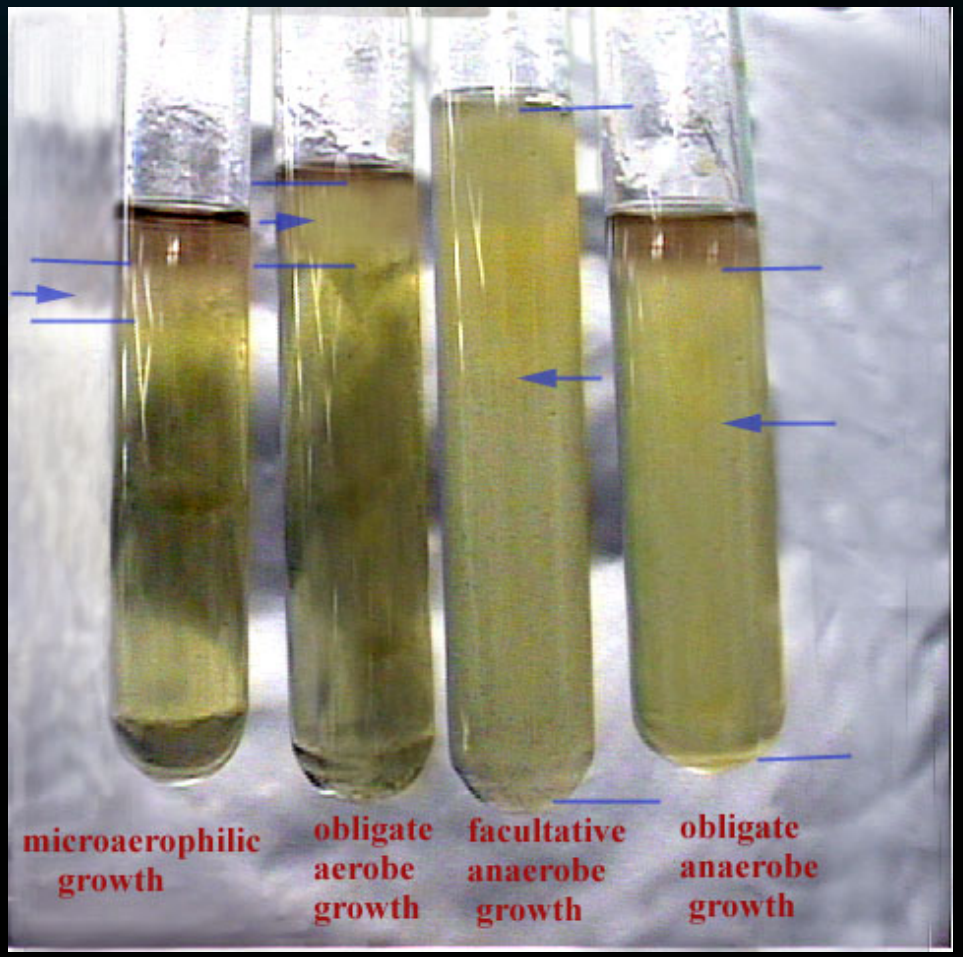
Inoculate (with loop) 4 thioglycollate tubes:

- *E. coli* (facultative anaerobe)
- *Clostridium sporogenes* (strict anaerobe)
- *Bacillus cereus* (facultative anaerobe)
- *Micrococcus luteus* (strict aerobe)

1) Aerobe
2) Obligate anaerobe
3) Micro-aerophile
4) Facultative anaerobe



RESULTS:



GAS PAK SYSTEM

- Uses an anaerobic jar which contains:

  1) inoculated plates to be grown anaerobically are placed inside

  2) gas generator envelope—produces H^+ and CO_2 upon the addition of water

  3) paladium catalyst—combines H^+ with oxygen to form H_2O

  4) indicator strip—impregnated with either methylene blue or resazurin to indicate whether anaerobic conditions inside the jar have been achieved

(The methylene blue indicator strip becomes colorless in absence of oxygen.)

zurin becomes pink in presence of oxygen.)

GasPak Anaerobic System

The **GasPak Anaerobic System** is used to create an oxygen-free environment for the growth of anaerobic microorganisms. Inoculated plates or tubes are placed inside the chamber, and anaerobic conditions are created by adding water to a **gas generator envelope** that is placed in the jar just before sealing.

The envelope contains two chemical tablets, **sodium borohydride** and **sodium bicarbonate**. Water reacts with these chemicals, producing **hydrogen gas** and **carbon dioxide**.

The hydrogen gas combines with free oxygen in the chamber to produce water, removing all free oxygen from the chamber. This reaction is catalyzed by the element **palladium**, which is attached to the underside of the lid of the jar. The **carbon dioxide** replaces the removed oxygen, creating a completely anaerobic environment.

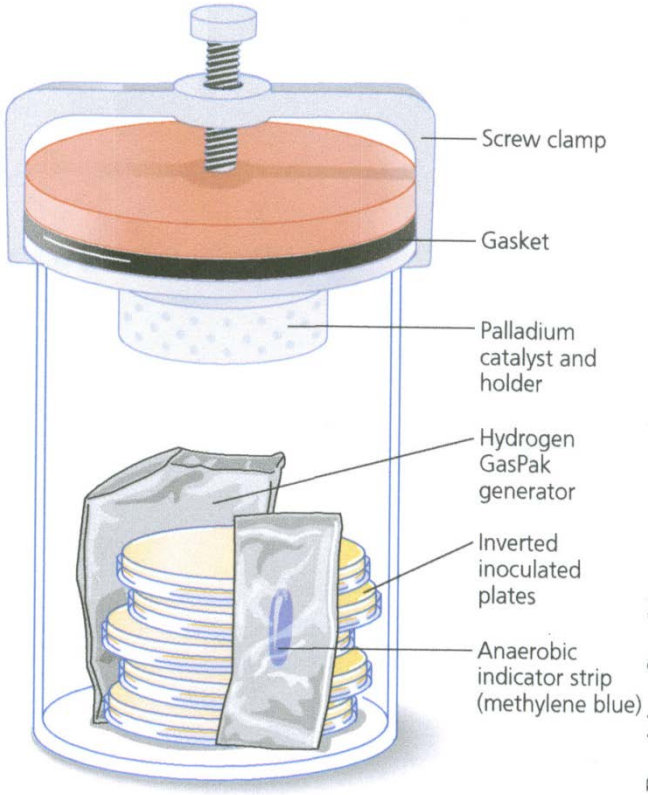
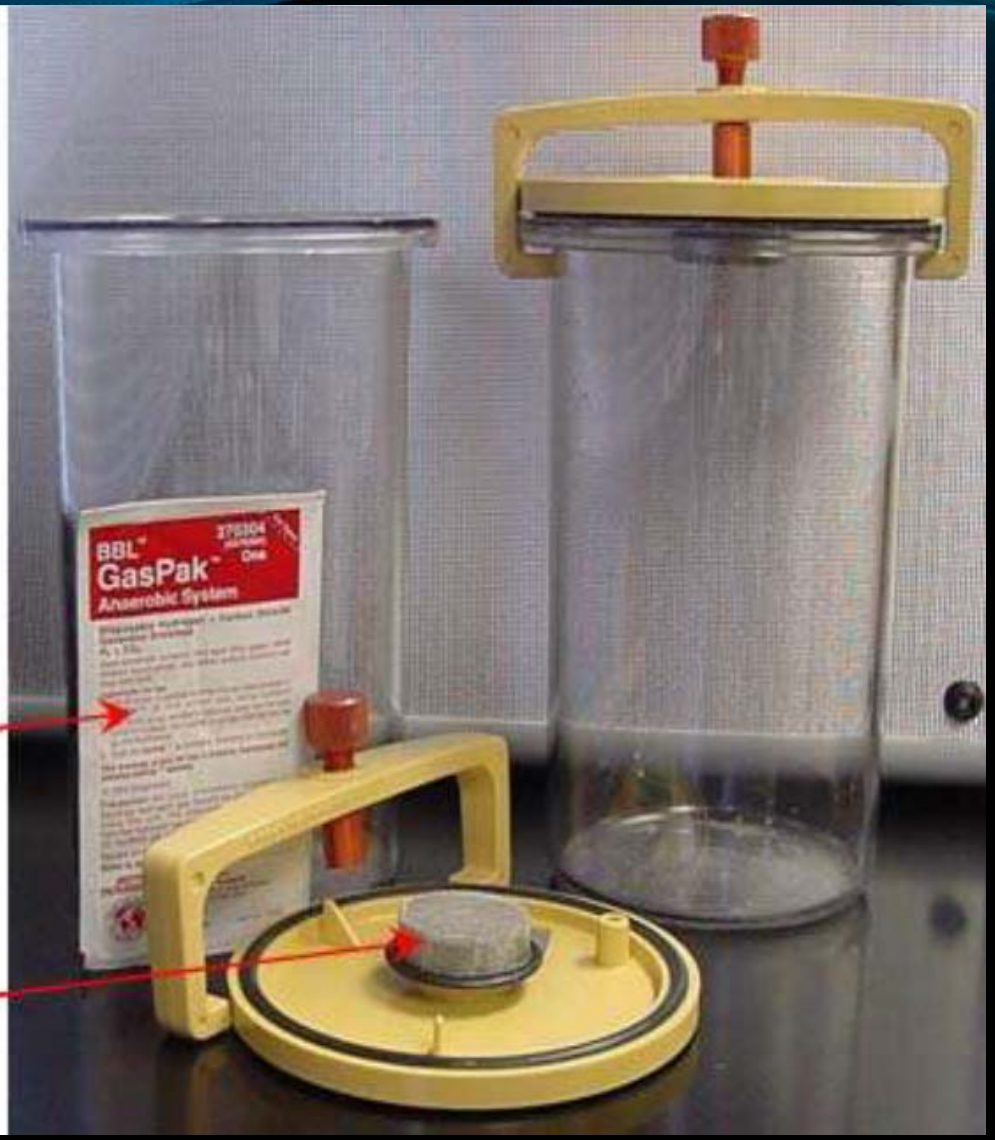


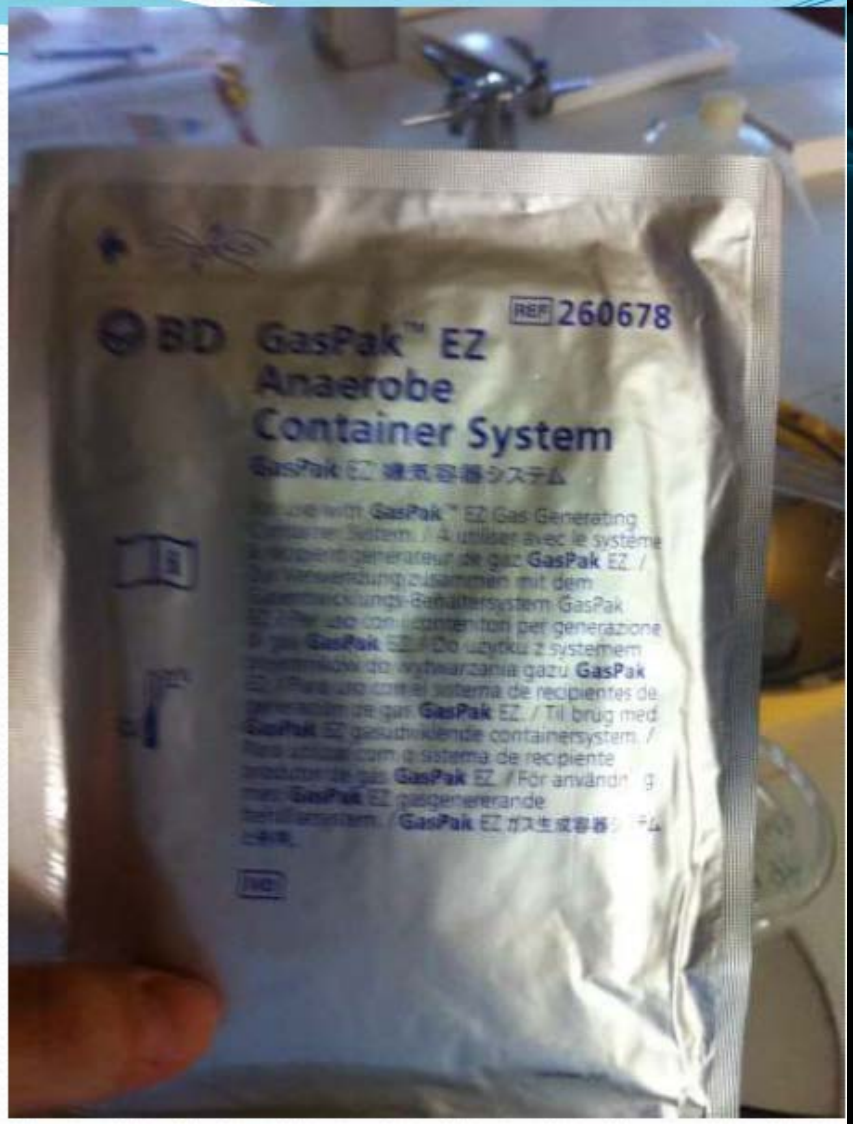
Figure 4 GasPak system



GasPak Envelope

Wire mesh containing palladium catalyst

- GasPak needing H_2O



- GasPak Not needing H_2O

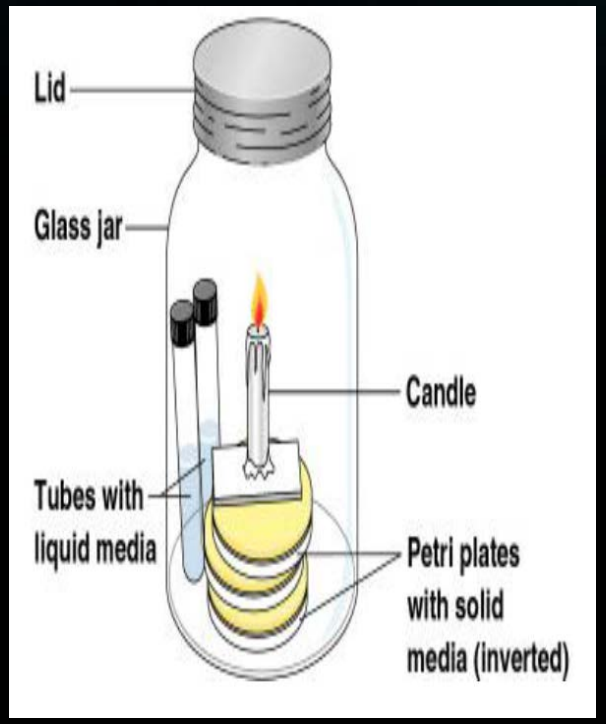
CANDLE JAR METHOD

Candle jar A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (20% concentration).

Many microphiles are also capnophiles, as they require an elevated concentration of carbon dioxide.

A candle jar is a container into which a lit candle is introduced before sealing the container's airtight lid .

The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar.



4- Mechanical exclusion of O2 (anaerobic incubator).

MICROSCOPY

Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye).

There are three well-known branches of microscopy:

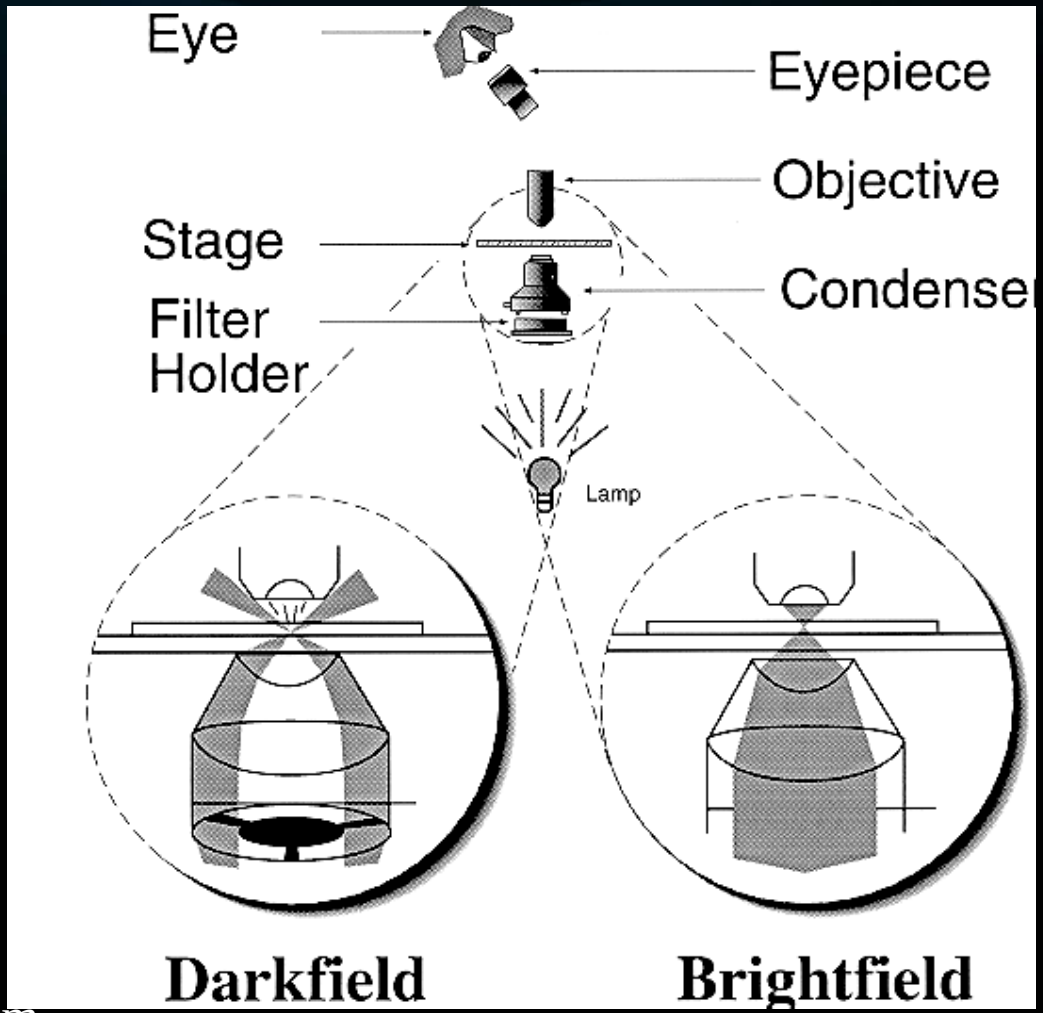
Optical

Electron

Scanning probe microscopy

along with the emerging field of X-ray microscopy.

BRIGHT FIELD AND DARKFIELD MICROSCOPY



- Optical and Electron microscopy measure refraction, diffraction, and reflection of the source radiation
  - Optical uses white light, fluorescent light, or lasers
  - Electron uses electromagnetic radiation/electron beams.
- Scanning uses a physical probe to interact with the surface of the specimen

Light microscopes

Most student microscopes are classified as **light microscopes**. In a light microscope, visible light passes through the specimen (the biological sample you are looking at) and is bent through the lens system, allowing the user to see a magnified image.

A benefit of light microscopy is that it can often be performed on living cells, so it's possible to watch cells carrying out their normal behaviors (e.g., migrating or dividing) under the microscope.

fluorescence microscopy

Another type of light microscopy is **fluorescence microscopy**, which is used to image samples that fluoresce (absorb one wavelength of light and emit another).

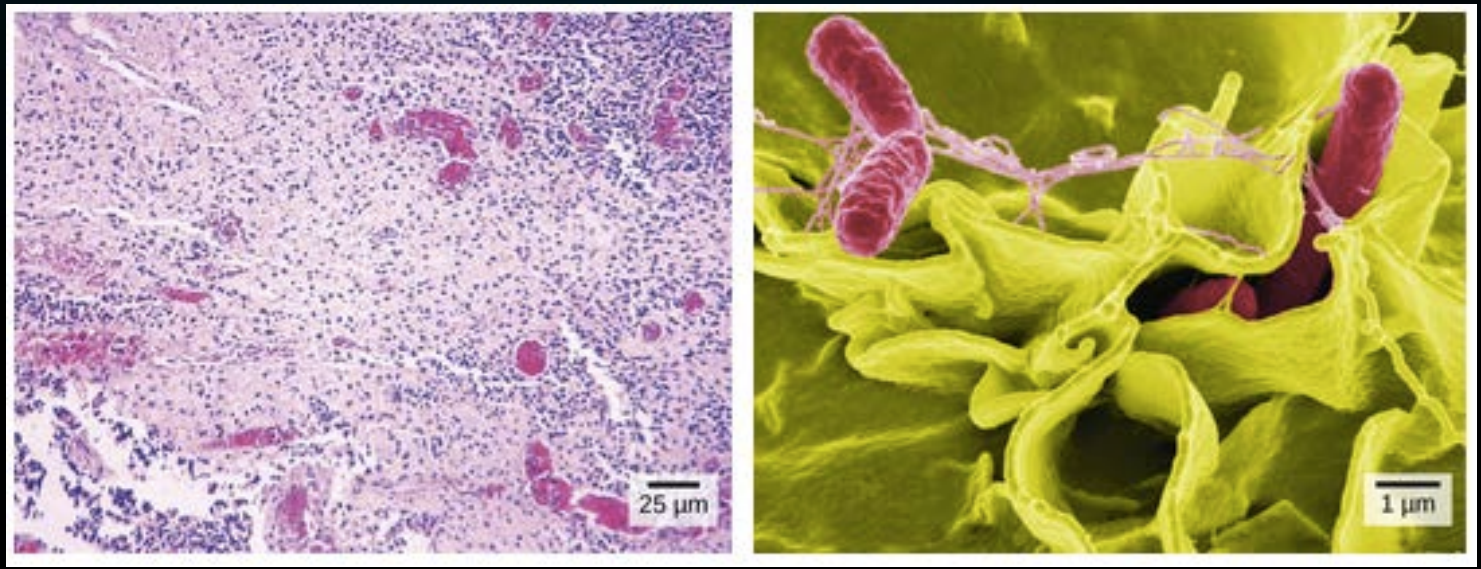
Light of one wavelength is used to excite the fluorescent molecules, and the light of a different wavelength that they emit is collected and used to form a picture. In most cases, the part of a cell or tissue that we want to look at isn't naturally fluorescent, and instead must be labeled with a fluorescent dye or tag before it goes on the microscope.

Electron microscopes

Some cutting-edge types of light microscopy (beyond the techniques we discussed above) can produce very high-resolution images. However, if you want to see something very tiny at very high resolution, you may want to use a different, tried-and-true technique: **electron microscopy**.

Electron microscopes differ from light microscopes in that they produce an image of a specimen by using a beam of electrons rather than a beam of light. Electrons have much a shorter wavelength than visible light, and this allows electron microscopes to produce higher-resolution images than standard light microscopes.

Electron microscopes can be used to examine not just whole cells, but also the subcellular structures and compartments within them.
One limitation, however, is that electron microscopy samples must be placed under vacuum in electron microscopy (and typically are prepared via an extensive fixation process). This means that live cells cannot be imaged.



In the image above, you can compare how *Salmonella* bacteria look in a light micrograph (left) versus an image taken with an electron microscope (right).

Electron microscope

There are two major types of electron microscopy.

- **Scanning electron microscopy (SEM)**
- **Transmission electron microscopy (TEM)**

In **scanning electron microscopy (SEM)**, a beam of electrons moves back and forth across the surface of a cell or tissue, creating a detailed image of the 3D surface. This type of microscopy was used to take the image of the *Salmonella* bacteria.

In **transmission electron microscopy (TEM)**, in contrast, the sample is cut into extremely thin slices (for instance, using a diamond cutting edge) before imaging, and the electron beam passes through the slice rather than skimming over its surface. TEM is often used to obtain detailed images of the internal structures of cells.