MICROBIAL GROWTH

Learning outcomes

1. List and explain the physical and chemical requirements for bacterial growth and development.
2. Explain the necessary criteria of a medium used for bacterial culture.
3. Distinguish reducing media, selective media, differential media and enrichment media in bacterial culture.
4. Prepare agar plates, agar deep and slants.
5. Obtain pure cultures

The Requirements for Growth

❑Physical Requirements

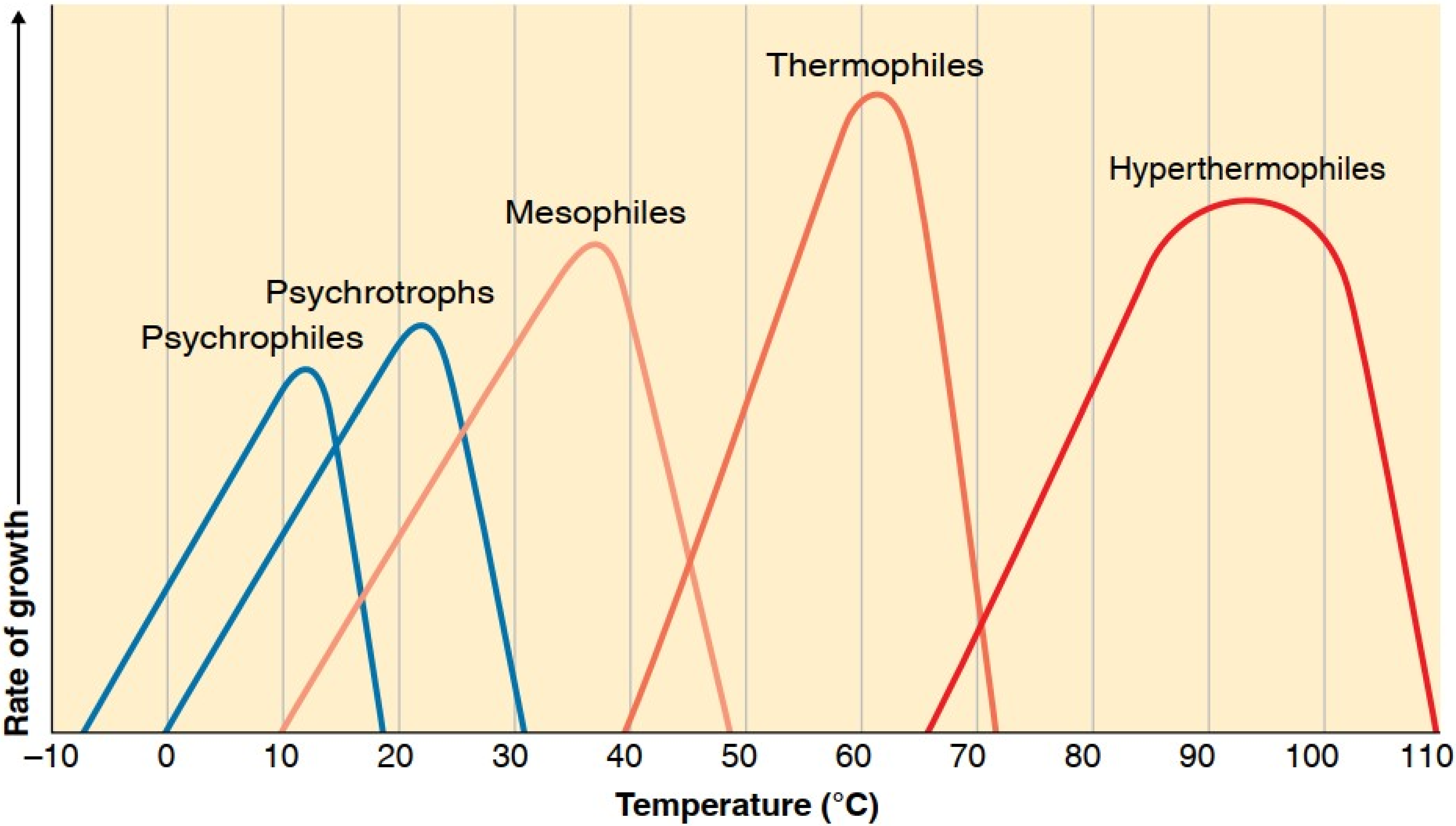
1. Temperature

- ✓ psychrophiles (cold-loving microbes)
- ✓ mesophiles (moderate-temperature–loving microbes)
- ✓ thermophiles (heat-loving microbes)

The Requirements for Growth

❑Physical Requirements

1. Temperature



The Requirements for Growth

❑ Physical Requirements

2. pH

- ✓ Most bacteria grow well at pH 6.5 - 7.5.
- ✓ Very few bacteria can grow at pH < 4.
- ✓ Some bacteria can still tolerate acid.
- ✓ (Most yeasts & molds grow well at pH 5 - 6).

=> The culture medium needs to be supplemented with chemical buffers: peptone, amino acids, phosphate salts ...

The Requirements for Growth

❑ Physical Requirements

3. Osmotic Pressure

- ✓ Bacteria require water for growth.

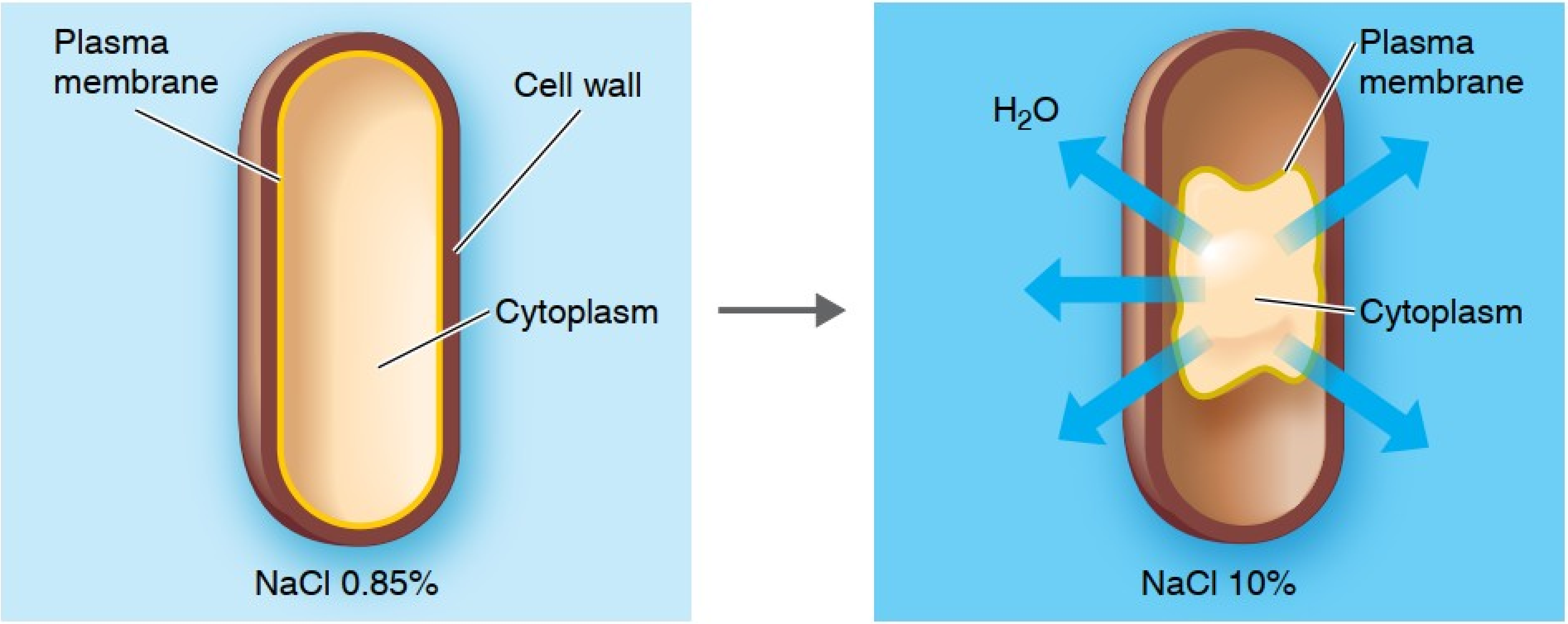
- ✓ High osmotic pressures have the effect of removing necessary water from a cell.

- ✓ When a microbial cell is in a solution whose concentration of solutes is higher than in the cell (the environment is hypertonic to the cell), the cellular water passes out through the plasma membrane to the high solute concentration. This osmotic loss of water causes plasmolysis, or shrinkage of the cell’s cytoplasm.

The Requirements for Growth

❑Physical Requirements

3. Osmotic Pressure



(a) Cell in isotonic solution. Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl).

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

The Requirements for Growth

❑ Physical Requirements

3. Osmotic Pressure

- ✓ Extreme halophiles require high salt concentrations for growth.

- ✓ Facultative halophiles, which do not require high salt concentrations but are able to grow at salt concentrations up to 2%, a concentration that inhibits the growth of many other organisms.

! A few species of facultative halophiles can tolerate even 15% salt.

The Requirements for Growth

❑Chemical Requirements

1. Carbon

- ✓ Besides water, carbon is one of the most important requirements for microbial growth.

- ✓ Carbon is the structural backbone of living matter; it is needed for all the organic compounds that make up a living cell.

- ✓ Half the dry weight of a typical bacterial cell is carbon.

The Requirements for Growth

❑ Chemical Requirements

1. Carbon

✓ Chemoheterotrophs get most of their carbon from the source of their energy - organic materials such as proteins, carbohydrates, and lipids.

✓ Chemoautotrophs and photoautotrophs derive their carbon from carbon dioxide.

The Requirements for Growth

TABLE 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

Characteristic	Eukaryotes	Prokaryotes		
	Algae, Plants	Cyanobacteria	Green Bacteria	Purple Bacteria
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Thylakoids in chloroplasts	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

The Requirements for Growth

TABLE 11.2 Selected Characteristics of Photosynthesizing Bacteria

Common Name	Example	Phylum	Comments	Electron Donor for CO_2 Reduction	Oxygenic or Anoxygenic
Cyanobacteria	*Anabaena*	Cyanobacteria	Plantlike photosynthesis; some use bacterial photosynthesis under anaerobic conditions	Usually H_2O	Usually oxygenic
Green nonsulfur bacteria	*Chloroflexus*	Chloroflexi	Grow chemoheterotrophically in aerobic environments	Organic compounds	Anoxygenic
Green sulfur bacteria	*Chlorobium*	Chlorobi	Deposit sulfur granules inside cells	Usually H_2S	Anoxygenic
Purple nonsulfur bacteria	*Rhodospirillum*	Proteobacteria	Can grow chemoheterotrophically as well	Organic compounds	Anoxygenic
Purple sulfur bacteria	*Chromatium*	Proteobacteria	Deposit sulfur granules inside cells	Usually H_2S	Anoxygenic

The Requirements for Growth

❑ Chemical Requirements

2. Nitrogen, Sulfur, and Phosphorus

- ✓ Necessary for the synthesis of cell components: proteins, DNA, RNA, ATP, phospholipids, etc.

- ✓ Many bacteria decompose protein-containing foods and other nitrogen-containing compounds; some other species obtain nitrogen from NH_4^+, NO_3^-, etc.
- ✓ Cyanobacteria use gaseous nitrogen (N_2) directly from the atmosphere. This process is called nitrogen fixation.

The Requirements for Growth

❑ Chemical Requirements

2. Nitrogen, Sulfur, and Phosphorus

- ✓ Some organisms that can use nitrogen fixation are free-living, mostly in the soil, but others live cooperatively in symbiosis with the roots of legumes such as clover, soybeans, alfalfa, beans, and peas. The nitrogen fixed in the symbiosis is used by both the plant and the bacterium.

The Requirements for Growth

❑Chemical Requirements

2. Nitrogen, Sulfur, and Phosphorus

- ✓ Sulfur is used to synthesize sulfur-containing amino acids and vitamins such as thiamine and biotin. Important sources of sulfur are SO_4^{2-}, H_2S, S-containing amino acids.

- ✓ Phosphorus is essential for the synthesis of nucleic acids and the phospholipids of cell membranes. Among other places, it is also found in the energy bonds of ATP. Important sources of phosphorus are PO_4^{3-}.

The Requirements for Growth

❑ Chemical Requirements

3. Trace Elements: **Fe^{2+}, Cu^{2+}, Mo^{2+}, Zn^{2+}, …**

- ✓ Besides K^{+}, Mg^{2+}, Ca^{2+}, etc. which are important factors for bacterial growth and are cofactors of enzymes, bacteria require very small amounts of these trace elements; Most are essential for the functions of certain enzymes, usually as cofactors.

- ✓ Although these trace elements are sometimes added to a laboratory medium, they are usually assumed to be naturally present in tap water and other components of media.

The Requirements for Growth

❑ Chemical Requirements

4. Organic Growth Factors

- ✓ Essential organic compounds an organism is unable to synthesize are known as organic growth factors; they must be directly obtained from the environment. One group of organic growth factors for humans is vitamins.

- ✓ Most vitamins function as coenzymes, the organic cofactors required by certain enzymes in order to function.

The Requirements for Growth

- **Chemical Requirements**

4. Organic Growth Factors

- ✓ Many bacteria can synthesize all their own vitamins and do not depend on outside sources. However, some bacteria lack the enzymes needed for the synthesis of certain vitamins, and for them those vitamins are organic growth factors. Other organic growth factors required by some bacteria are amino acids, purines, and pyrimidines.

The Requirements for Growth

❑ Chemical Requirements

5. Oxygen

- ✓ Bacteria that use molecular oxygen produce more energy from nutrients than bacteria that do not.

- ✓ Bacteria that require molecular oxygen for life are obligate aerobes.

- ✓ Facultative anaerobes can use oxygen when it is present but are able to continue growth by using *fermentation* or *anaerobic respiration* when oxygen is not available.

The Requirements for Growth

❑Chemical Requirements

5. Oxygen

- ✓ Anaerobes are bacteria that are unable to use molecular oxygen for energy-yielding reactions. In fact, most are harmed by it.
- ✓ Aerotolerant anaerobes are fermentative and cannot use oxygen for growth, but they tolerate it fairly well (lactic acid bacteria have superoxide dismutase which neutralizes superoxide free radicals).

- ✓ Microaerophiles are aerobic but they do require a small amount of oxygen (less than the amount found in air) for growth.

TABLE 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; growth ceases in presence of oxygen.	Only anaerobic growth; but growth continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

The Requirements for Growth

❑Chemical Requirements

5. Oxygen

- Toxic forms of oxygen

✓ Singlet oxygen ($\cdot O_2^-$) is normal molecular oxygen (O_2) that has been boosted into a higher-energy state and is extremely reactive.

The Requirements for Growth

❑ Chemical Requirements

5. Oxygen

- Toxic forms of oxygen

✓ Superoxide radicals (O^{2-}), or superoxide anions, are formed in small amounts during the normal respiration of organisms that use oxygen as a final electron acceptor, forming water.

- In the presence of oxygen, obligate anaerobes also appear to form some superoxide radicals, which are so toxic to cellular components that all organisms attempting to grow in atmospheric oxygen must produce an enzyme, superoxide dismutase (SOD), to neutralize them.
- Their toxicity is caused by their great instability, which leads them to steal an electron from a neighboring molecule, which in turn becomes a radical and steals an electron, and so on.

The Requirements for Growth

❑ Chemical Requirements

5. Oxygen

- Toxic forms of oxygen

✓ Superoxide radicals (O^{2-}), or superoxide anions, are formed in small amounts during the normal respiration of organisms that use oxygen as a final electron acceptor, forming water.

$$O_2^- + O_2^- + 2\,H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

$$H_2O_2 + 2\,H^+ \xrightarrow{\text{Peroxidase}} 2\,H_2O$$

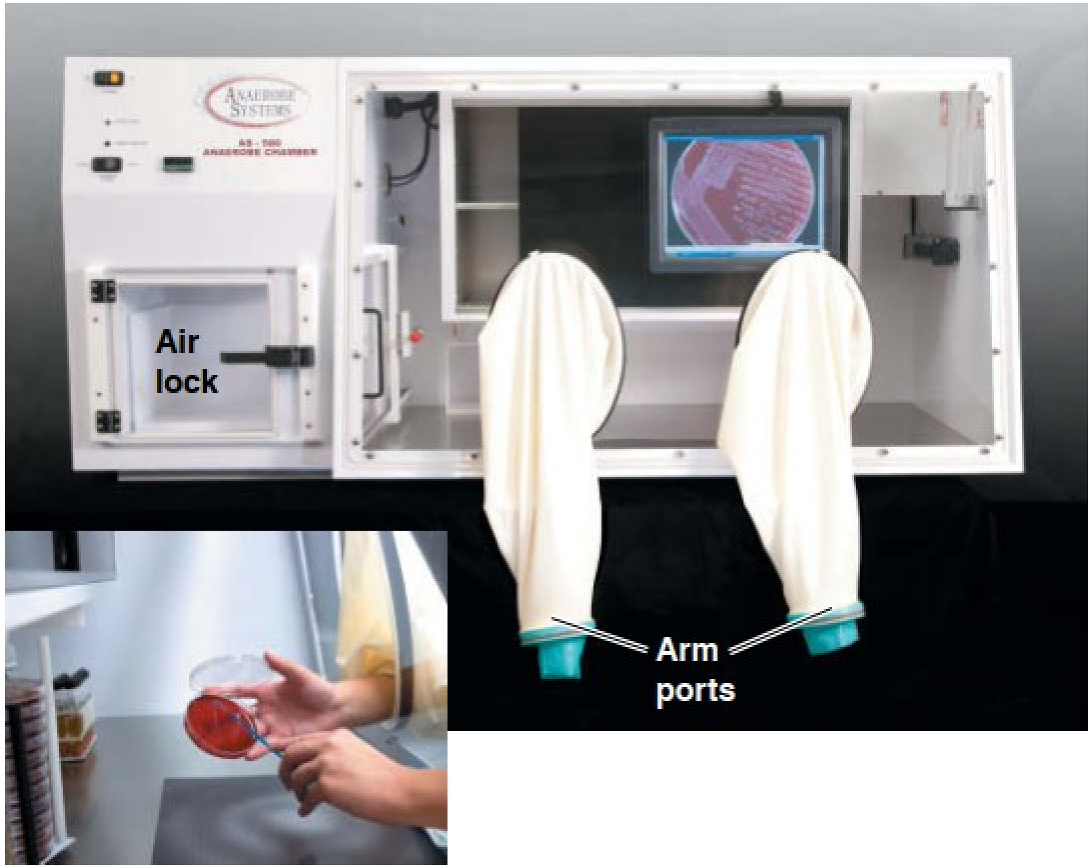
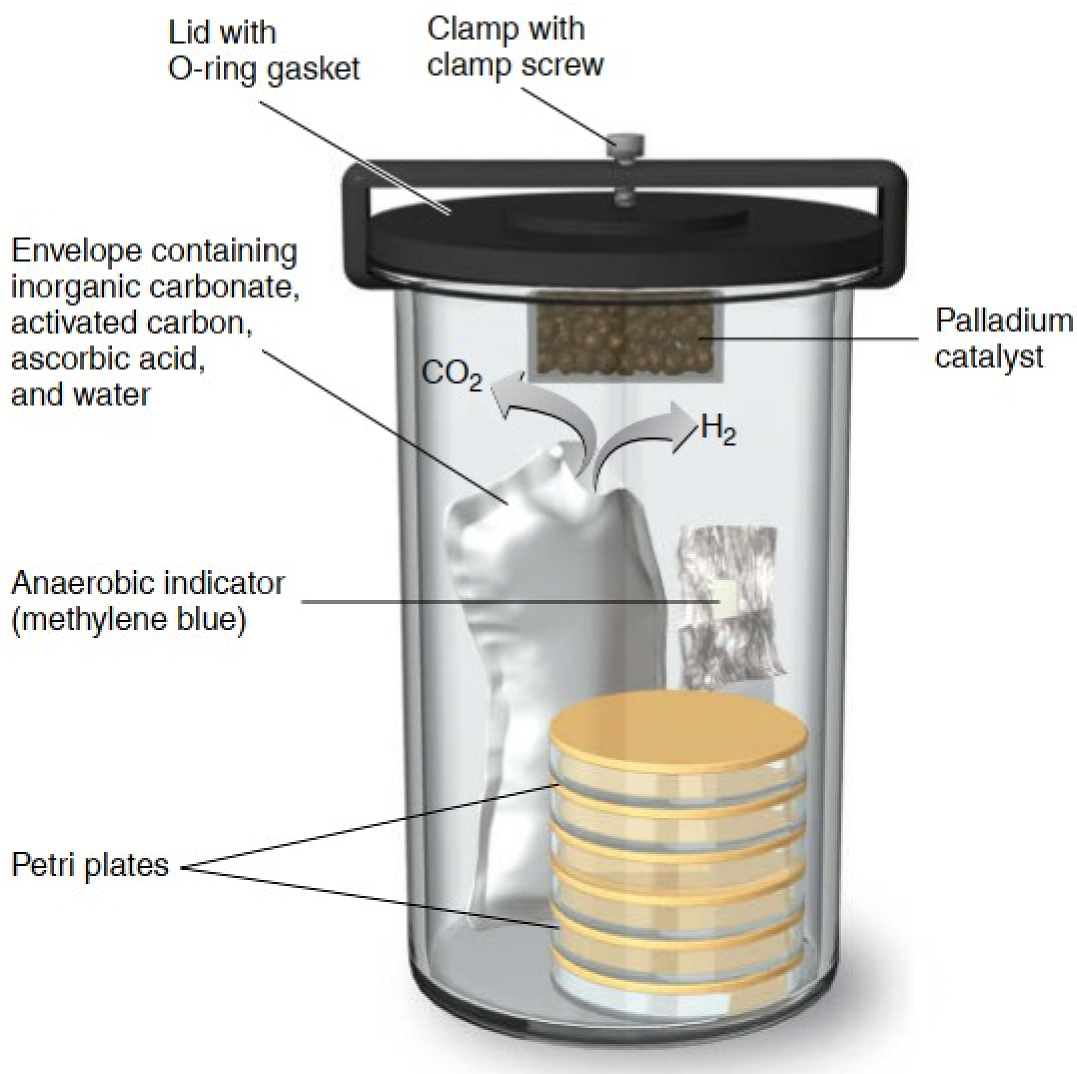
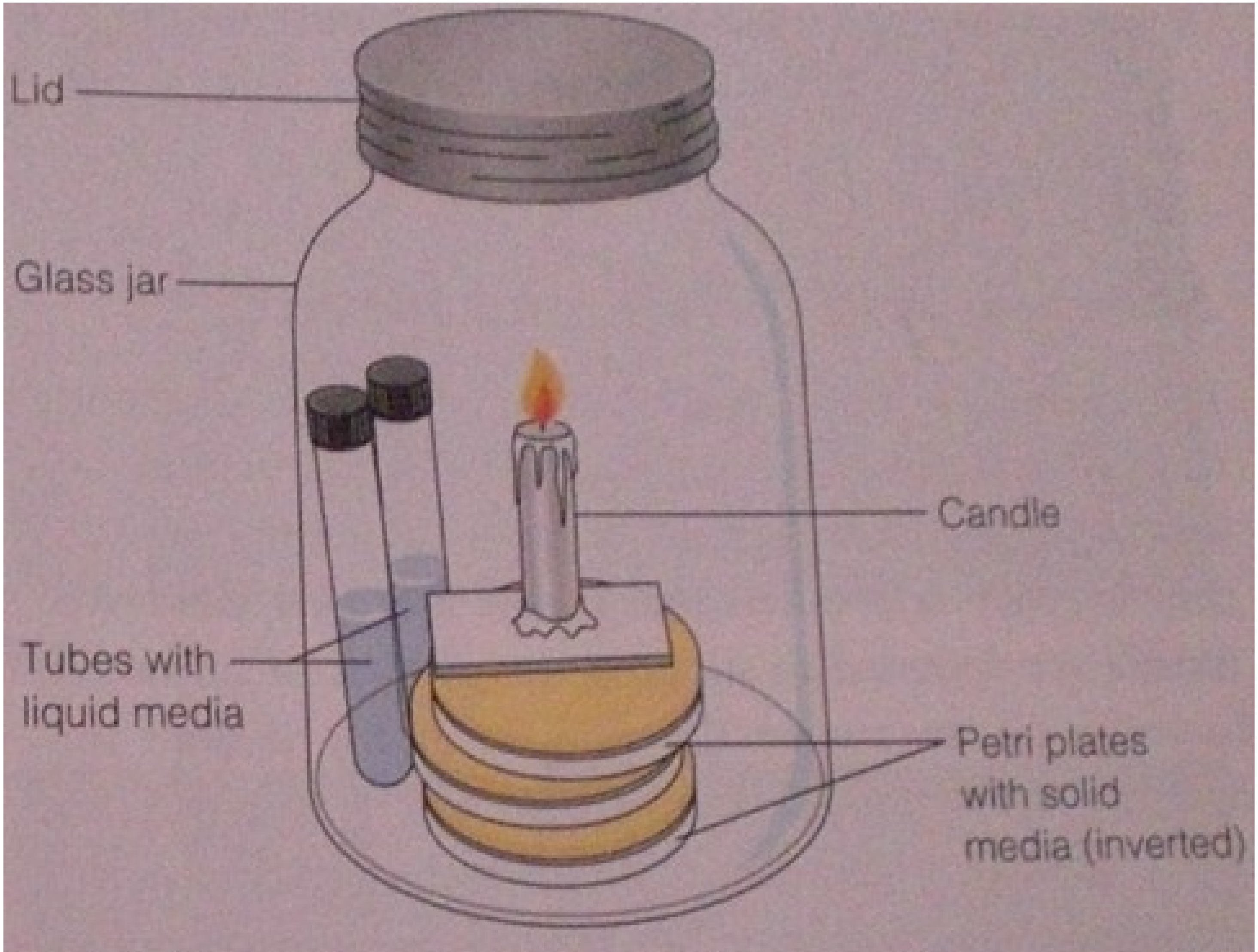


Figure 6.7 An anaerobic chamber. Materials are introduced through the small doors in the air-lock chamber at the left. The operator works through arm ports in airtight sleeves. The airtight sleeves extend into the cabinet when it is in use. This unit also features an internal camera and monitor.

Figure 6.8 Technicians in a biosafety level 4 (BSL-4) laboratory. Personnel working in a BSL-4 facility wear a "space suit" that is connected to an outside air supply. Air pressure in the suit is higher than the atmosphere, preventing microbes from entering the suit.



$$NaHCO_3 + NaBH_4 + H_2O \rightarrow H_2 + CO_2$$


Lid
Glass jar
Candle
Tubes with
liquid media
Petri plates
with solid
media (inverted)

(a) Candle jar

The Requirements for Growth

!!! Other microorganisms also have similar physical & chemical requirements as the bacteria.

❑ Culture Media

- A culture medium is an environment that contains nutrient materials prepared for the growth of microorganisms in a laboratory.

- Criteria of a culture medium:
  - ✓ must contain the right nutrients for the specific microorganism
  - ✓ should contain sufficient moisture, a properly adjusted pH, and a suitable level of oxygen
  - ✓ must initially be sterile
  - ✓ the growing culture should be incubated at the proper temperature

Culture Media

- Microbes that are introduced into a culture medium to initiate growth are called an inoculum.

- The microbes that grow and multiply in or on a culture medium are referred to as a culture.

❑ Culture Media

- Ready-to-use media: just add water and sterilize.
- Agar is a complex polysaccharide derived from a marine alga (seaweed). It is a solidifying agent. It melts at ~100 ^{o}C but remains liquid until < 40^{o}C.

  - ✓ Deep agar
  - ✓ Slant
  - ✓ Agar plate

Culture Media

Chemically Defined Media

A chemically defined medium is one whose exact chemical composition is known.

TABLE 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

Culture Media

Complex Media

- A complex medium is made up of nutrients including extracts from yeasts, meat, or plants, or digests of proteins from these and other sources. The exact chemical composition varies slightly from batch to batch.

TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Culture Media

Reducing media

Reducing media contain ingredients, such as sodium thioglycolate, that chemically combine with dissolved oxygen and deplete the oxygen in the culture medium.

❑ Culture Media

❖ Selective media

Selective media are designed to suppress the growth of unwanted bacteria and encourage the growth of the desired microbes.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	5 g
Beef Extract	5 g
Dextrose	5 g
Disodium Phosphate	4 g
Ferrous Sulfate	0.3 g
Bismuth Sulfite Indicator	8 g
Brilliant Green	0.025 g
Agar	20 g

Final pH: 7.5 ± 0.2 at 25°C

Salmonella Typhi

Bismuth Sulfite agar (modified Wilson Blair)

❑ Culture Media

❖ Differential media

Differential media make it easier to distinguish colonies of the desired organism from other colonies growing on the same plate.

Formula / Liter

Enzymatic Digest of Casein .... 15 g
Enzymatic Digest of Animal Tissue .... 4 g
Yeast Extract .... 2 g
Corn Starch .... 1 g
Sodium Chloride .... 5 g
Agar .... 14 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

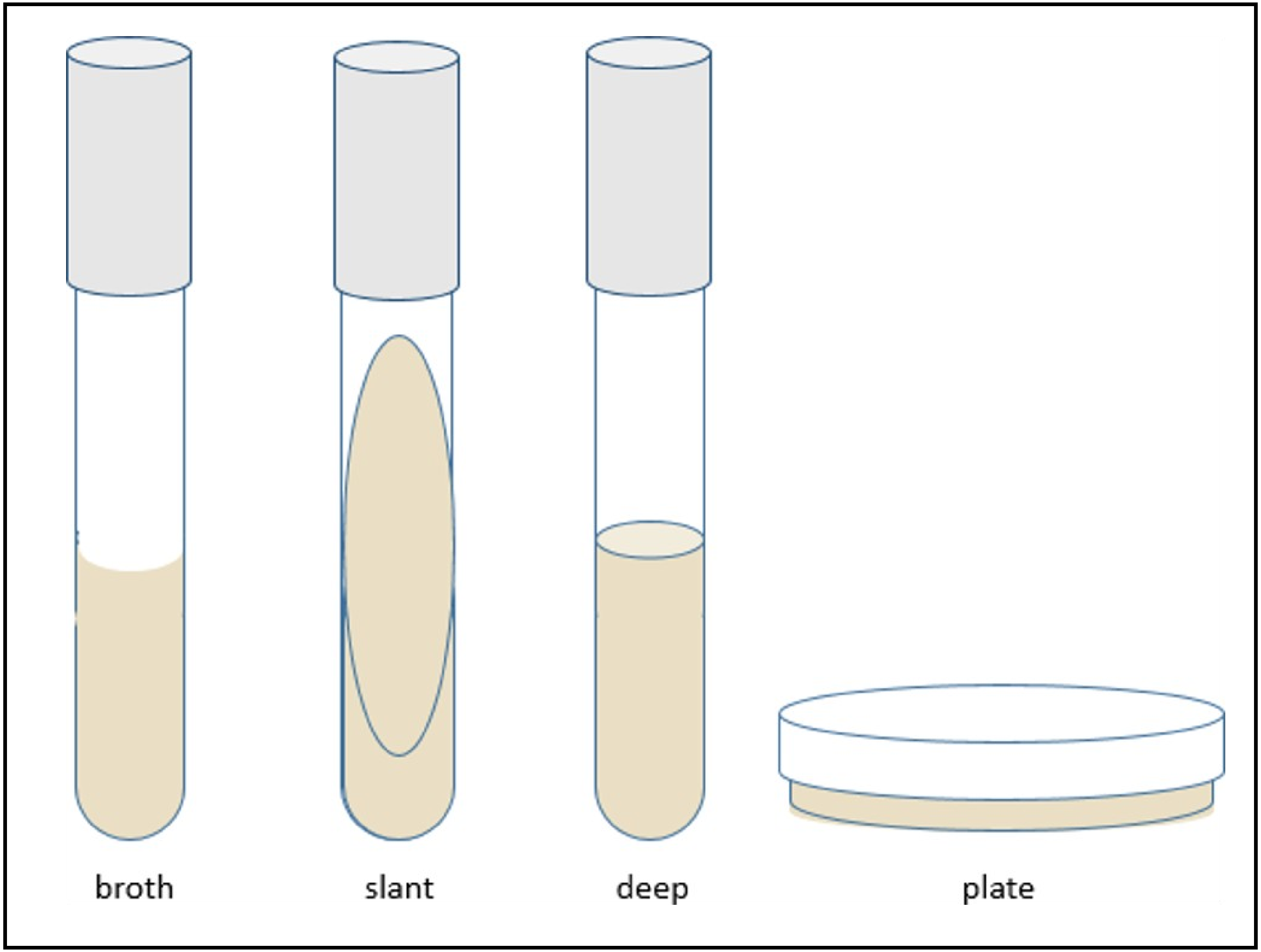
Culture Media

Enrichment media

An enrichment culture is a culture whose concentration is enriched due to the microbes are initially present in small numbers.

The enrichment medium for an enrichment culture is usually liquid and provides nutrients and environmental conditions that favor the growth of a particular microbe but not others. In this sense, it is also a selective medium, but it is designed to increase very small numbers of the desired type of organism to detectable levels.

Culture Media



Obtaining Pure Cultures

Colony

A visible colony theoretically arises from a single spore or vegetative cell or from a group of the same microorganisms attached to one another in clumps or chains.

❑ Obtaining Pure Cultures

❖ Streak plate method