Lecture 4

Microbial Nutrition

Microorganisms, like all organisms, have a basic requirement for several essential elements for growth. These are necessary for the biosynthesis of the components of cells as well as their function. **Nutrition** is a process by which chemical substances called **nutrients** are acquired from the environment and used in cellular activities such as metabolism and growth.

The common nutrients requirement

Analysis of microbial cell composition show that 95% or more of cell dry weigh is made up of few major elements (carbon, nitrogen, oxygen, hydrogen, Sulphur, phosphorus, magnesium, potassium, sodium, calcium and iron). The first four are called as **macronutrients**, while the rest are called as Micronutrients, or **trace elements.** Carbon has a pivotal role in the structure of all molecules found in living cells, however, nitrogen, hydrogen, oxygen, sulphur and phosphorus are all essential as well. Of less significance, in respect of the amount required, are many trace nutrients which are predominantly metal ions e.g. Zn, Cu, Mn etc.

An inorganic nutrient is an atom or simple molecule that contains a combination of atoms other than carbon and hydrogen. The natural reservoirs of inorganic compounds are mineral deposits in the crust of the earth, bodies of water, and the atmosphere. Examples include metals and their salts (magnesium sulfate, ferric nitrate, sodium phosphate), gases (oxygen, carbon dioxide), and water. In contrast, the molecules of organic nutrients contain carbon and hydrogen atoms and are usually the products of living things. They range from the simplest organic molecule, methane (CH4), to large polymers (carbohydrates, lipids, proteins, and nucleic acids). Micronutrients are all metal ions, and frequently serve as cofactors for enzymes

Carbon

Carbon is the central component of the biological macromolecules. Carbon incorporated into biosynthetic pathways may be derived from organic or inorganic sources; some organisms can derive it from CO_2, while others require their carbon in ‘ready-made’, organic form.

Hydrogen

Hydrogen is a major element in all organic and several inorganic compounds, including water, salts and gases (H_2S, CH_4, and H_2). These

gases are both used and produced by microbes. Hydrogen performs these overlapping roles in the biochemistry of cells:

1. Maintaining **pH,**

2. Forming **hydrogen bonds** between molecules, and

3. Serving as the source of **free energy** in oxidation-reduction reactions of respiration.

Oxygen

Oxygen is a major component of organic compounds such as carbohydrates, lipids, nucleic acids, and proteins, it plays an important role in the structural and enzymatic functions of the cell. Oxygen is likewise a common component of inorganic salts such as sulfates, phosphates, nitrates, and water.

Nitrogen

Nitrogen is needed for the synthesis of proteins and nucleic acids, DNA, RNA as well as for important molecules such as ATP. Some bacteria and algae utilize inorganic nitrogenous nutrients (NO_3 _, NO_2_, or NH_3). A small number of prokaryotes can transform N_2 into compounds usable by other organisms through the process of nitrogen fixation. Regardless of the initial form in which the inorganic nitrogen enters the cell, it must first be

converted to NH_3, the only form that can be directly combined with carbon to synthesize amino acids and other compounds.

Sulphur

Sulphur is required for the synthesis of proteins and vitamins, and in some types is involved in cellular respiration and photosynthesis. It may be derived from Sulphur containing amino acids (methionine, cysteine), sulphates and sulphides.

Phosphorus

The main inorganic source of phosphorus is phosphate (PO_4 _), derived from phosphoric acid (H_3PO_4) and found in rocks and oceanic mineral deposits. Phosphate is a key component of nucleic acids and is thereby essential to the genetics of cells and viruses.

Metals such as copper, iron and magnesium are required as *cofactors* in enzyme reactions. Many microorganisms are unable to synthesise certain organic compounds necessary for growth and must therefore be provided with them in their growth medium. These are termed *growth factors*, of which three main groups can be identified: amino acids, purines and pyrimidines (required for nucleic acid synthesis) and vitamins.

Other important elements in microbial metabolism include mineral ions. Potassium is essential to protein synthesis and membrane function.

Sodium is important for certain types of cell transport. Calcium is a stabilizer of the cell wall and endospores of bacteria. Magnesium is a component of chlorophyll and a stabilizer of membranes and ribosomes. Iron is an important component of the cytochrome proteins of cell respiration. Zinc is an essential regulatory element for eukaryotic genetics.

Classification of Bacteria Based on Nutritional Requirement

A- According to carbon source:

1- **Autotrophic:** is an organism that uses inorganic CO_2 as its carbon source. Because autotrophs have the special capacity to convert CO_2 into organic compounds, they are not nutritionally dependent on other living things.

2- **Heterotrophic:** is an organism that must obtain its carbon in an organic form. Because organic carbon originates from the bodies of other organisms, heterotrophs are dependent on other life forms. Among the common organic molecules that can satisfy this requirement are proteins, carbohydrates, lipids, and nucleic acids.

B- According to energy source:

1- **Phototrophic:** microbes use light as an energy source, such as *Chlorobium*, *Chromatium* and *Rhodospirillum*.

2- **Chemotrophic:** microbes obtain energy from the oxidation of chemical compounds.

C-According to the electron source:

1- **Lithotrophs:** microbes reduce inorganic substances an electron source.

2- **Organotrophs:** microbes extract electrons or hydrogen from organic compounds.

Photolithotrophic autotrophs (often called phototrophs): MO use light energy and Co_2 as a carbon source.

Chemoorganotrophic heterotrophs (often called chemotrophs or heterotrophs): MO use organic compounds as a source of energy, hydrogen, electron and carbon for biosynthesis.

Nutrition	Energy source	Carbone source	e.g
Photolithotrophic	Light	Co_2	Eukaryotic algae and cyanobacteria
Photoorganotrophic	Light	organic	purple , green bacteria
Chemolithotrophic	inorganic compounds H_2, NH_3, NO_2, H_2S	CO2	*Nitrosomonas, Nitrobacter, Thiobacillus*
Chemoorganotrophic	organic compounds as sources of energy, hydrogen, electrons, and carbon	carbon	Pathogenic bacteria

Lecture 5 the Study of Microbial Growth

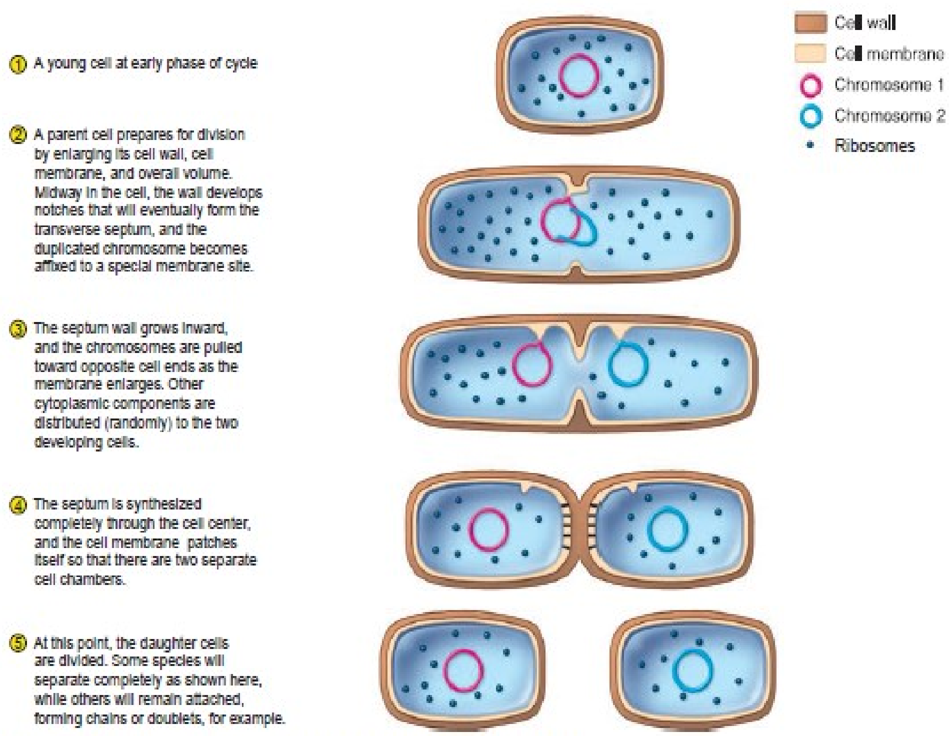
When microbes are provided with nutrients and the required environmental factors, they become metabolically active and grow. Growth takes place on

two levels. On one level, a cell synthesizes new cell components and increases its size; on the other level, the number of cells in the population increases. This capacity for multiplication, increasing the size of the population by cell division, has tremendous importance in microbial control, infectious disease, and biotechnology. Growth in unicellular microorganisms such as bacteria, yeasts and protozoans, however, is more properly defined in terms of an increase in the size of a given *population*. This may be expressed as an increase in either the number of individuals or the total amount of *biomass*. In other words it is an increase in all the cell components, which ends in multiplication of cell leading to an increase in population.

Cell Division

The division of a bacterial cell occurs mainly through **binary fission,** or **transverse fission;** *binary* means that one cell becomes two, and *transverse* refers to the division level forming across the width of the cell. During binary fission, the parent cell enlarges, duplicates its chromosome, and forms a central transverse septum that divides the cell into two daughter cells. This process is repeated at intervals by each new daughter

cell in turn, and with each successive round of division, the population increases.



Process Figure 7.13 Steps in binary fission of a rod-shaped bacterium.

Phases of Growth

Consider a population of organisms introduced into a fresh, nutrient-rich **medium**, a mixture of substances on or in which microorganisms grow. Such organisms display four major phases of growth: (1) the lag phase, (2) the log (logarithmic) phase, (3) the stationary phase, and (4) the decline phase, or death phase. These phases form the **standard bacterial growth curve** (**Figure**).

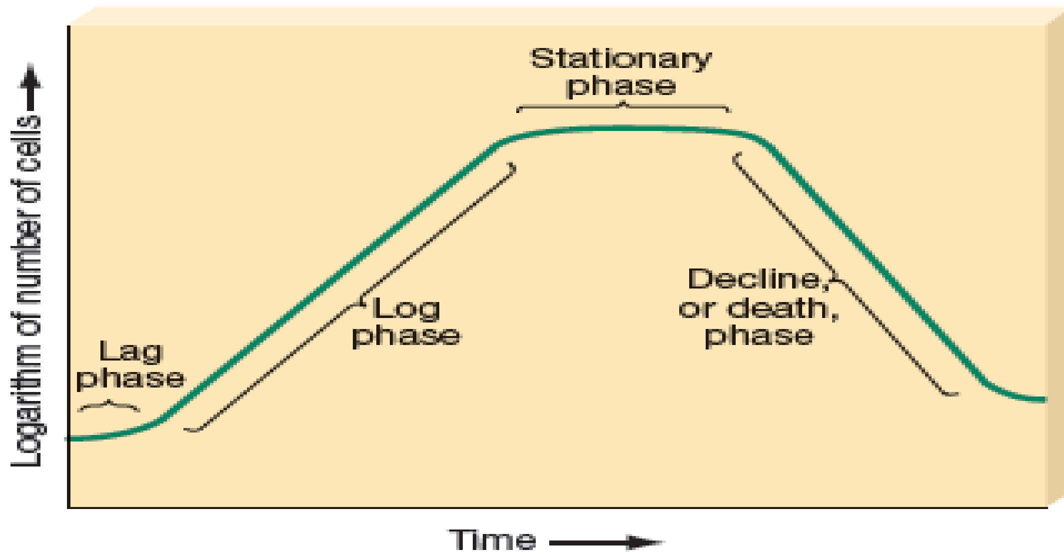
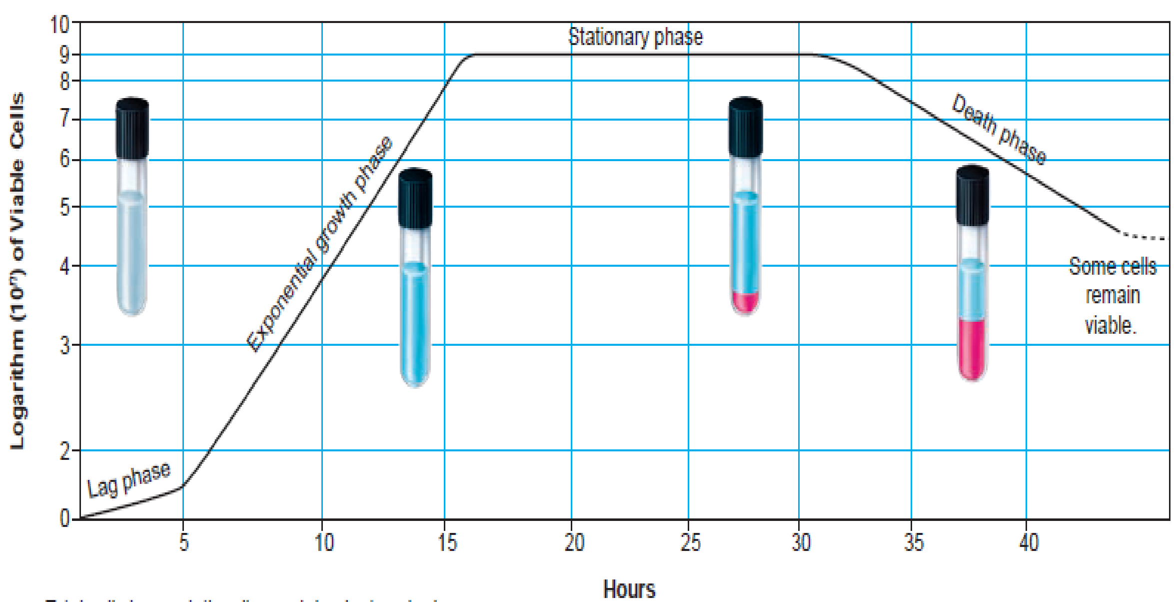


FIGURE 6.3 A standard bacterial growth curve.



1- **<u>The lag phase</u>**

When an inoculum of bacteria is first introduced into some growth medium, it will probably require a period to adapt to its new surroundings – the less familiar these are, the longer the period of adaptation. If, for example, the carbon source in the medium is unfamiliar, the cells will need time to synthesise the necessary enzymes for its metabolism. The length of the lag phase will also depend on the age and general health of the cells in the inoculum. During this period, there is no net increase in bacterial numbers, however the cells are metabolically active.

2- **The log (exponential) phase**

Once organisms have adapted to their new environment and synthesised the enzymes needed to utilise the available substrates, they are able to start regular division by binary fission. This leads to the exponential increase in numbers referred to above. Under optimal conditions, the population of cells will double in a constant and predictable length of time, known as the *generation (doubling) time*. This phase will continue as long as cells have adequate nutrients and the environment is favorable.

3- **The stationary phase**

When cell division decreases to the point that new cells are produced at the same rate as old cells die, the number of live cells stays constant. The culture is then in the **stationary phase**, represented by a horizontal straight line in Figure. The medium contains a limited amount of nutrients and may contain toxic quantities of waste materials. Also, the oxygen supply may become inadequate for aerobic organisms, and damaging pH changes may occur.

4- <u>**The decline death phase**</u>

As conditions in the medium become less and less supportive of cell division, many cells lose their ability to divide, and thus the cells die. In this decline phase, or death phase, the number of live cells decreases at a logarithmic rate, as indicated by the straight, downward-sloping diagonal line in Figure. During the decline phase, many cells undergo *involution*—that is, they assume a variety of unusual shapes, which makes them difficult to identify. In cultures of spore-forming organisms, more spores than vegetative (metabolically active) cells survive. The duration of this phase is as highly variable as the duration of the logarithmic growth phase. Cultures of some bacteria go through all growth phases and die in a few days; others contain a few live organisms after months or even years.

Generation time

During the log phase, the organisms divide at their most rapid rate—a regular, genetically determined interval called the **generation time**. The population of organisms doubles in each generation time. For example, a culture containing 1,000 organisms per milliliter with a generation time of 20 minutes would contain 2,000 organisms per milliliter after 20 minutes, 4,000 organisms after 40 minutes, 8,000 after 1 hour, 64,000 after 2 hours, and 512,000 after 3 hours. Such growth is said to be *exponential*, or *logarithmic*.

Calculate generation time

In order to calculate the generation time the following formula is always used.

$$N_t = N_o \times 2^n$$

Where N_t is the final cell number

N_o is the original cell number

n is the number of generation

$$Generation\ time = \frac{final\ time}{division\ number}$$

$$\text{the number of division} = \frac{the\ final\ time}{generation\ time}$$

Question what is the final number of the growing cells knowing that the original cell number is 100 cells and the division number is 3?

The final cell number = the original cell number x 2^n

The final cell number = the original cell number× 2^3

The final cell number = 800 bacterial cell

Question what the final number of growing cells is during three hours knowing that the original number is 100 and the generation time is 15 minutes.

First we need to convert hours into minutes

3×60= 180 minutes

$$\text{the number of division} = \frac{the\ final\ time}{generation\ time}$$

$\text{the number of divison} = \frac{180}{15}$ = 12

The final cell number = 100 × 2^{12}

The final cell number = 100 × 2^2 × 2^5 × 2^5

The final cell number = 100×4096

The final cell number= 409600 bacterial cell

Table 6.1	An Example of Exponential Growth			
Time[a]	Division Number	2^n	Population ($N_o \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

Batch culture and continuous culture

continuous culture

Sometimes it is desirable to keep the culture in the logarithmic phase, for example if the cells are being used to produce alcohol or antibiotics. In a *continuous culture*, nutrient concentrations and other conditions are held constant, and the cells are held in a state of exponential growth. This is achieved by continuously adding fresh culture medium and removing equal volumes of the old. Parameters such as pH can also be monitored and adjusted. The equipment used to do this is called a chemostat it produces a steady-state culture whose population size is kept constant by careful control of flow rates and nutrient concentrations.

Batch culture

The phases of growth described above apply to a *batch culture*. In this form of culture, appropriate nutrients and other conditions are provided for growth, then an inoculum is added and the culture incubated. No further nutrients are added and no waste products are removed, thus conditions in the culture are continually changing.

Measuring Bacterial Growth

Bacterial growth is measured by estimating the number of cells that have arisen by binary fission during a growth phase. This measurement is expressed as the number of *viable* (living) organisms per milliliter of culture.

Several methods of measuring bacterial growth are available:

- **Direct methods – count individual cells**
- **Indirect Methods – measure effects of bacterial growth**

- **Direct methods**
  - Serial dilution and viable plate counts
  - Membrane filtration
  - Most probable number
  - Microscopic counts
  - Electronic counters

- **Indirect methods**
  - Metabolic activity
  - Dry weight
  - Turbidity

Spectrophotometer-measures amount of light that passes through a sample. Absorbance is related to the number of bacteria