Cultivation of Microorganisms

Cultivation of microorganisms Cultivation is the process of propagating organisms by providing the proper environmental conditions. Organisms require metabolic energy to synthesize macromolecules and to maintain essential chemical gradients across their membranes. Additionally, nutrients must be provided in metabolically accessible form.

Microorganisms vary widely in their nutritional demands and their source of metabolic energy. Factors that must be controlled during growth include the nutrients, PH, temperature, aeration, salt concentration, & ionic strength of the medium. The three major mechanisms for generating metabolic energy are fermentation, respiration & photosynthesis. At least one of these mechanisms must be employed if an organism is to grow.

Culture Techniques

Requirements for growth

Like all living organisms , microorganisms require an energy source , usually in the form of an organic carbon compound , and a range of other nutrients for metabolism and cell growth . Microorganisms can be divided into two main groups according to the source of energy utilized. **Prototrophs** use light as an energy source , whereas **chemotrophs** use different chemical substances as their source of energy . Many microorganisms use organic substances as energy sources, although some are able to use inorganic substances. These are both different types of chemotrophs

, but those which use organic substances as energy sources are known as **chemoorganotrophs** , whereas those which use inorganic substances are referred to as **chemolithotrophs** .

The nutrients which are required by microorganisms can be divided into two groups:

- **Macronutrients**, which are required in relatively large amounts
- **Micronutrients**, which are required in small quantities.

Macronutrients include carbon, hydrogen, oxygen, nitrogen, sulphur, magnesium and iron (Table : l). All nutrients have to be provided in a suitable form in the culture media in which the microorganisms are grown. Carbon is often provided in the form of organic substances, including glucose, organic acids, fatty acids or amino acids. Phototrophic microorganisms, such as ***Chlorella***, use carbon dioxide as their carbon source. Nitrogen is available to microorganisms either in organic substances, such as amino acids or nucleotide bases, or as inorganic substances including ammonia or nitrate ions. Many bacteria are able to use ammonia as their only source of nitrogen; the nitrogen fixing bacteria, such as ***Rhizobium***, use nitrogen gas.

Table : 1 Macronutrients required by microorganisms and the forms in which they are supplied in culture media

Elements	Forms in which the elements are supplied in culture media
Carbon (C)	Glucose , organic acid , yeast extract , peptone
Hydrogen (H)	Water , organic compound
Oxygen (O)	Water , oxgen gas , organic compounds
Nitrogen (N)	Nitrogen gas ammonium ions , nitrate ions , amino acid nucleotide bases

Phosphorus (P)	Inorganic phosphates
Sulphur (S)	Sulphates , sulphur – containing amino acids
Magnesium (Mg)	Magnesium salts such as magnesium sulphate
Sodium (Na)	Sodium chloride
Calcium (Ca)	Calcium chloride
Iron (Fe)	Iron salts such as iron sulphate
Potassium (K)	Potassium salts such as potassium chloride

Micronutrients (or trace elements) are metals and are essential for normal cell function. They are required in very small quantities and it is not normally necessary to add these separately to culture media as they will often be present in sufficient quantities in other ingredients. Micronutrients include copper, manganese, vanadium and zinc. These may function as enzyme activators or as constituents of enzyme molecules. Iron is sometimes considered to be a micronutrient, although it is required in larger quantities than the other metals.

In addition to these micronutrients, microorganisms may also require certain organic growth factors in very small amounts. Such factors include amino acids, vitamins, purines and pyrimidines. These compounds can be synthesized by the majority of microorganisms, but some microorganisms may require one or more of these to be present in their culture media.

All the nutrients required by microorganisms must be provided in the media in which they are grown. There are two main types of culture media used in microbiology, defined and undefined (or complex) media. **Defined media** are made up using pure chemical substances, dissolved in distilled water so that the exact chemical composition is known. **Undefined media** contain mixtures of substances such as yeast extract, peptone, or casein hydrolysate, in which the exact composition is

unknown. To illustrate these types of culture media, Table 2. shows examples of culture media for *Escherichia coli*.

Table 2.: Examples of culture media for *E. coli*

Defined culture medium for ***E. coli***	Un defined culture medium for ***E. coli***
K_2HPO_4	Glucose
KH_2PO_4	Yeast extract
$(NH_4)_2SO_4$	Peptone
$MgSO_4$	KH_2PO_4
$CaCl_2$	Distilled water
glucose	
Distilled water	

Environmental influences on growth

We have described the nutrients which are required by microorganisms for growth, but there are several other factors which have important influences on growth. These factors include **temperature**, availability of **oxygen**, **light** (for phototrophic microorganisms) and **pH.** Temperature is one of the most important factors. In general, as temperature increases, enzyme activity within the cells also increases and growth becomes faster. However, above a certain temperature, proteins - including enzymes will be denatured. Therefore, growth will increase up to a point above which enzymes become denatured and inactivated. Above this point, growth rate falls rapidly to zero. For every microorganism, there is a **minimum** temperature, below which there is no growth, an **optimum** temperature where

growth occurs at the most rapid rate and **a maximum** temperature above which growth will not occur.

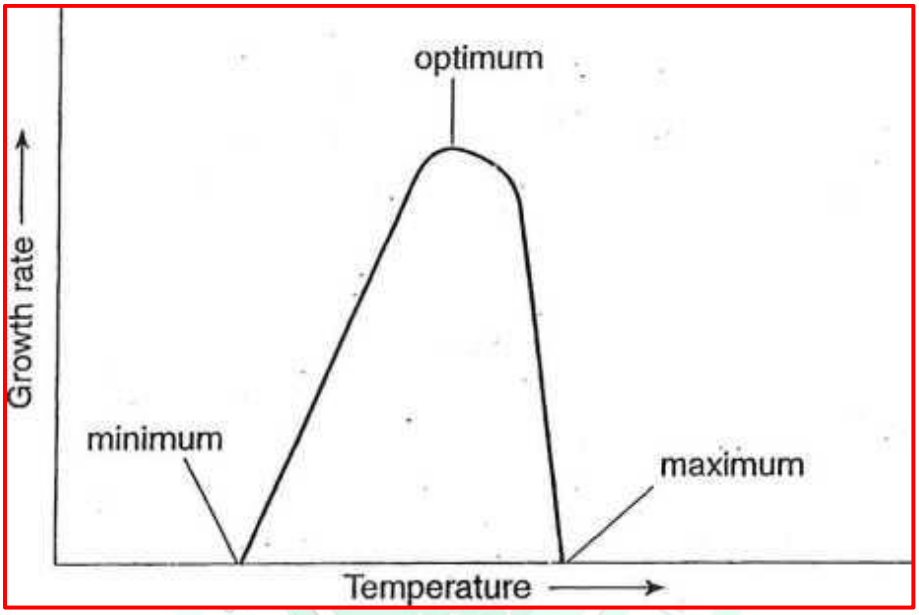

Figure : The effect of temperature on the growth rate of a microorganism