

UNIT-IV

Microbial assay



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- Principles and methods of different microbial assay

Introduction

- The microbiological or microbial assay is a type of **biological assay in which the relative potency of activity of a compound is determined** by measuring **the amount required for producing the predicted effect** on a suitable test organism under standard conditions.
- The microbiological assay is a biological assay performed using microorganisms, e.g., **bacteria, yeast, and moulds**.
- These assays are much easier than those performed using mammals for isolating essential vitamin; for example, a growth factor for *Lactobacillus* proved to be similar to a hematopoietic factor in pernicious anemic patients.

- Many therapeutic agents, such as **antibiotics** inhibiting microbial growth or the essential growth factors (**vitamins** and **amino acids**), are **standardized by microbiological assays**.
- The activity of antibiotics (**extent of inhibiting the microbial growth**), vitamins, or amino acids (**extent of supporting the microbial growth**) is determined by **microbiological assays**; while the **potency (concentration or amount)** of such substances is determined by **chemical assays**.
- Thus, microbiological assays of antibiotics, vitamins, and amino acids are significantly important.

Principles

- A microbiological assay relies on the principle that when **certain compounds are present in limited amounts, the amount of microbial growth corresponds to the amount of these compounds.**
- The basic procedure of most of the microbial assays is same; however, the test conditions vary.
- The **test substance is added to a liquid or gel medium, test microorganism is inoculated on the medium, and the resultant response (which depends on the substance's biochemical effect on the test organism) is observed.**

- It may be a **growth response (positive in the assay of nutrients and negative in the assay of antibiotics)**, which is determined **by counting, optical density, weight, or area**; the **growth response** may be a definite **end-point, or an all-or none response**.
- In case of either a positive or negative metabolic response, the metabolic products or changes in some function may be measured. Examples of some **measurable metabolic responses** are **acid production, CO₂ production, oxygen uptake, nitrate reduction, haemolysis of RBCs, anti luminescent activity, or inhibition of spore germination**.
- Substances **analogous and antagonistic to regular metabolites inhibit the growth of wild, prototrophic strains of bacteria**. The **nutrilite content** can be assayed by adding **graded amounts of nutrilit** for **re-activating the growth**.

Merits: A microbiological assay has the following merits:

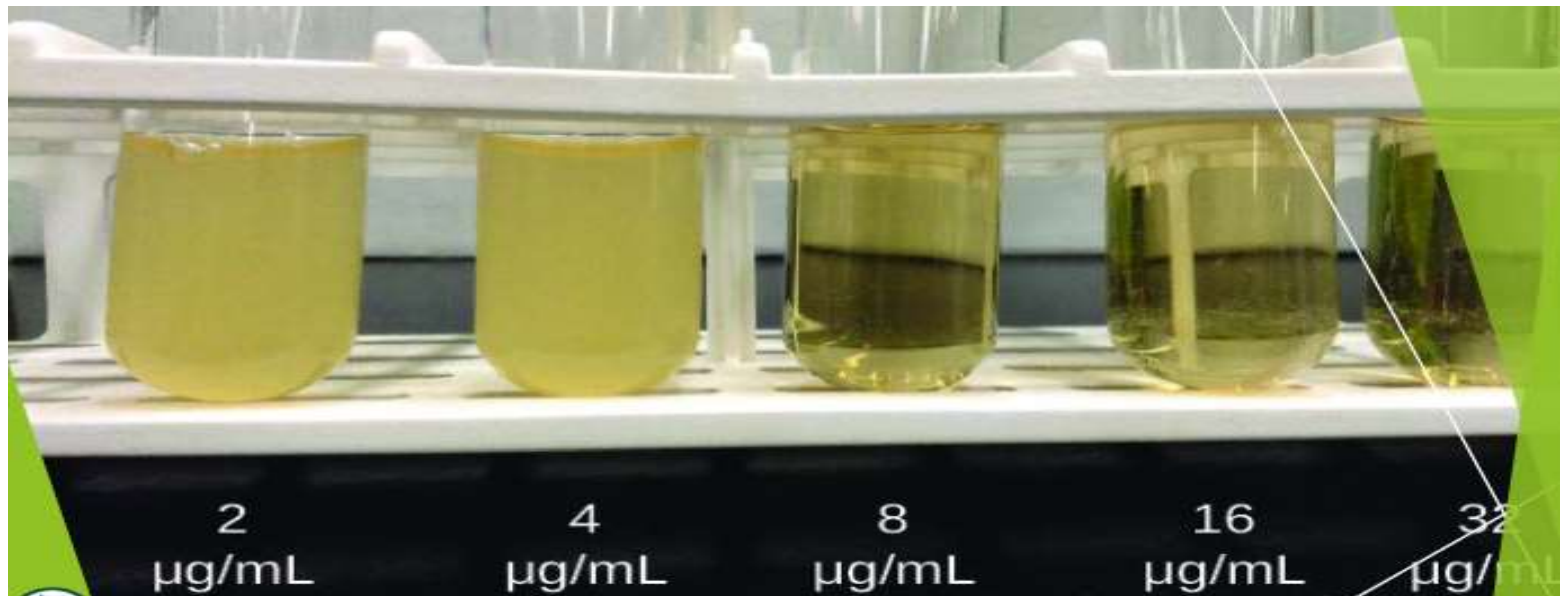
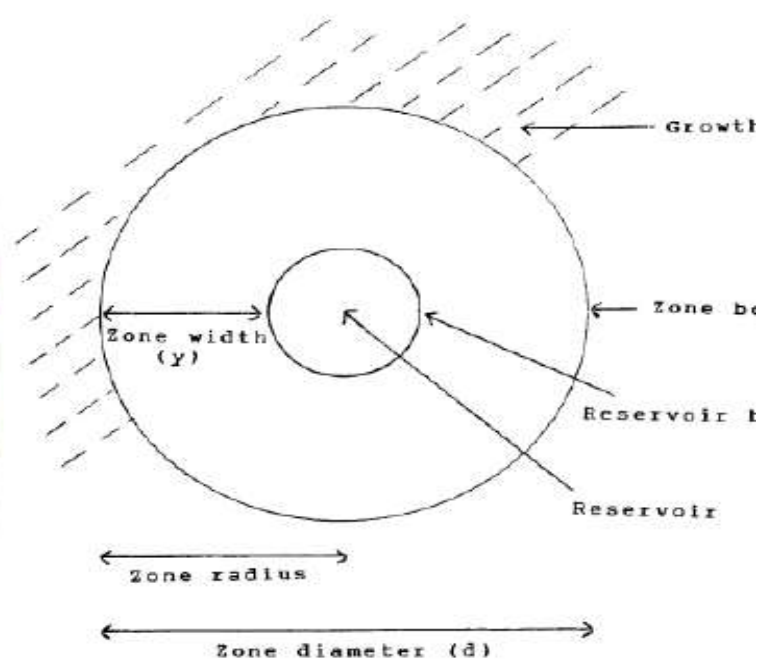
- 1) It is **suitably used** for compounds which cannot be assayed by either **physical or chemical methods**.
- 2) It is used for the assay of **naturally occurring therapeutic agents**.
- 3) It **minimizes the mortality rate of animals**.
- 4) It is a **simple and rapid method** as compared to **bioassays**.
- 5) It is used for **accurate standardization of medicinal compounds**.
- 6) It **determines the concentration** as well as **activity of** compounds.
- 7) It **does not require large amount of sample** and instruments.
- 8) Its complete procedure can be automated to minimize the duration.

Demerits: A microbiological assay has the following demerits:

- 1) It requires a **specific test organism** for the assay of a **particular compound**.
- 2) It **demands maintenance of sterile conditions** within the laboratory.
- 3) It **may give invalid results** due to a **slight variation in incubation temperature**.
- 4) It is a **time-consuming method**.
- 5) It requires **well trained and expert individuals**.

Standardization of Antibiotics/ Microbiological Assay of Antibiotics

- The microbiological assay of an antibiotic is based upon a **comparison of the inhibition of growth of micro-organisms by measured concentrations of the antibiotics under examination with that produced by known concentrations of a standard preparation of the antibiotic having a known activity.**
- Two general methods are usually employed, i.e.,
 - **Cylinder-plate (or cup plate) method**
 - **Turbidometric (or tube assay) method.**



- The **cylinder-plate method** (Method A) depends upon **diffusion of the antibiotic from a vertical cylinder through a solidified agar layer** in a Petri dish or plate to an extent such that **growth of the added micro-organism is prevented entirely in a zone around the cylinder containing a solution of the antibiotic.**
- The **Turbidometric method** (Method B) depends upon the **inhibition of growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic.**