Oxidase enzyme

The oxidase test is a biochemical reaction that used for detection to the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase.

In the final stage of bacterial respiration involves a series of membrane embedded components which known as the electron transport chain, the chain may involve the use of the enzyme cytochrome oxidase, which catalyzes the oxidation of cytochrome c while reducing oxygen to form water. The oxidase test often uses a reagent, When the reagent is oxidized by cytochrome c, it changes from colorless to a dark blue or purple compound, indophenol blue .

The method of work:

1. Put a small drops of 1% oxidase reagent on filter paper.

2. Transport by Using a loop a well-isolated colony from a fresh bacterial plate (18- to 24 hour culture) to treated filter paper .

3. Observe for color changes.

4. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds while Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

physical factors

- **Effect of Oxygen**

Microbes are classified according to their need for oxygen to produce energy into four major groups:

1- Strict aerobes microorganisms: These microorganisms require the presence of oxygen in sufficient concentration for their growth.
2- Strict anaerobes microorganisms: These microorganisms lives and active under anaerobic conditions.
3- Facultative anaerobes: this group of microorganisms lives in the presence or absence of oxygen.
4- Microaerophilic microorganisms: These microorganisms require a oxygen but low concentration less than concentration for growth Strict aerobes.

The method of work: (Deep agar)

1. Dip a straight, sterile inoculation needle into the bacterial suspension.
2. We inoculate the test tubes containing the deep nutrient agar environment by the lancing method.
3. The tubes were incubated at 37 ° C for 48 hours.
4. After the incubation period ends, we observe the resulting bacterial growth and then determine the bacteria's need for oxygen based on the following information and the figure:

a. If growth is only on the surface of the environment, the bacteria are considered obligatory.

b. If the growth is at the end of the lancing line (below the tube) the bacteria are considered Strict anaerobes.

c. If the growth is along the acupuncture line with the needle pathway the bacteria are considered anaerobic optionally.

d. If the growth is near the surface of the environment the bacteria are considered to be of the low oxygen demand (approximately 0.5-1 cm below the surface of the environment).

- **Effect of PH degree**

- Microbial growth is affected by changes in the degree of hydrogen ion concentration in the developing environment. Microbial growth stops at high acidity and alkalinity, which affect enzyme activity and metabolism.
- For each microbial species (Optimum degree) of hydrogen ion concentration, the growth is the largest possible. And (Maximum degree), which is the maximum degree at which growth occurs. Each microbial species has a (minimum degree), which is the degree to which if the concentration of hydrogen ion decreases, growth stops completely.

  Microbes are divided according to their tolerance to hydrogen ion concentration into:

  1. Acidophilic microorganism
  2. Acidotolerant microorganism
  3. Neutrophilic microorganism
  4. Alkalophilic microorganism
  5. Alkalotolerant microorganism

The method of work:

1. Inoculate nutrient agar plates Which have different pH concentrations (9 - 7.2 - 5.5 - 4.5) with bacteria.
2. The plates are incubated inverted at 37 ° C for 48 hours.
3. Record the results for each microbial species.

- **Effect of temperature:**

1. **The effect of low (Minimum) temperature on bacteria:** When the temperature decreases, all vital activities become very slow because the viscosity of the protoplasm increases, and consequently the kinetic energy of the enzyme decreases and its activity decreases. It also affects the role that the cell membrane plays in transporting nutrients to and from the cell, which in turn leads to a decrease in the cell's metabolic activity**.**
2. **The effect of high (Maximum) temperature on bacteria:** All biochemical reactions stop as a result of changing the nature of the enzyme begins with the deadly effect of the heat and the denaturation of proteins include enzyme**.**
3. **The effect Optimum temperature on bacteria:** Stimulating all biochemical reactions in the microbe to an optimum level**.**

<u>The method of work:</u>

1- 6 plates from nutrient agar are inoculated with the tested bacteria, then each plate is incubated at a different temperature as follows (5, 10, 28, 37, 42, 55 degrees) for 48 hours, after incubation examined growth it.

2- Note in the table the results related to bacterial growth as follows

No growth = - , weak growth = +, medium growth = + +, prolific growth+ + + = .

3- Draw a curve showing the relationship between different temperatures and the amount of growth for each microbial species.

4- Determine the maximum, optimum and minimum temperature for each microbial species.