Lab 6 Hydrogen ion concentration (pH)

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

$$pH=-\log [H^+]= \log \left(\frac{1}{[H+]}\right)$$

It is not surprising that pH dramatically affects microbial growth. Each species has a definite pH growth range and pH growth optimum:

Acidophiles : have their growth optimum between pH 0 and 5.5.

Neutrophiles : Their growth optimum between pH 5.5 and pH 8.

Alkalophiles : They prefer the pH range of 8 to 11.5.

Extreme alkalophiles : have their growth optimum at pH 10 or higher.

In general different microbial groups have characteristic pH preferences.

Most bacteria are neutrophiles. Although microorganisms will often grow over wide ranges of pH and far from their optimum, there are limits to their tolerance, and variations in cytoplasmic pH can harm M.O. that cause disruption of plasma membrane or inhibit the activity of enzymes and membrane transport proteins. Changes in the external pH also might alter the ionization of nutrient molecules and reduce their availability to the organisms.

Microorganisms respond to external pH changes using mechanisms that maintain a neutral cytoplasmic pH. Several mechanisms for adjusting to small changes in external pH, the plasma membrane is impermeable to proton. Neutrophiles appear to exchange potassium for protons using membrane transport system, alkalophiles maintain their internal pH closer to neutrality by exchanging internal pH closer to neutrality by exchanging internal sodium ions for external protons.

Other pH maintaining mechanism employed by different bacterial types termed Decarboxylation and Deamination which occurs in periplasmic space by involving free amino acids, where pH drops. Carboxyle groups

splits from free amino acids resulting in elevation of pH again, in case of pH increase to alkaline region. Deamination technique operate in periplasm that split amino groups from free amino acids which reduced pH value.

Microorganisms frequently change the pH of their own habitat particularly in batch culture system by producing acidic or basic metabolic waste products.

Because M.O. change the pH of their surrounding, buffers often are included in culture media to prevent growth inhibition by large pH changes. Phosphate buffer is a commonly used buffer, buffering capacity of this buffer related by a weak acid ($H_2PO^-_4$)and its conjugate base(HPO^-_4)

$H^+ + HPO_4^{2-} \longrightarrow H_2PO^-_4$

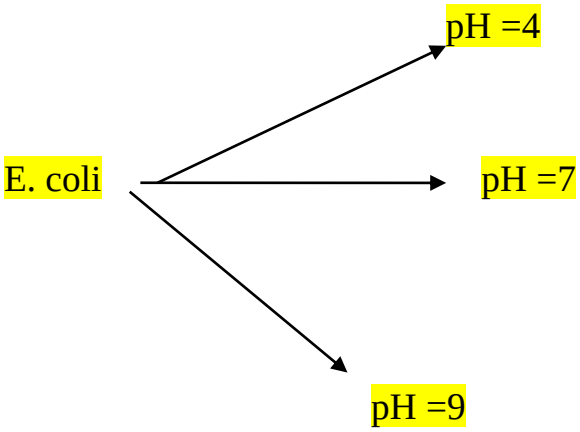
$OH^- + H_2PO^-_4 \longrightarrow H_2PO_4^{2-} + HOH$

If protons are added to the mixture, they combine with the salt from to yield a weak acid. An increase in alkalinity is resisted because the weak acid will neutralize hydroxyl ions through proton donation to give water, peptides and amino acids in complex media also have a strong buffering effect.

pH =1 such as bacteria *Thiobacillus thiooxidans*(Acidophiles)

pH=11 such as bacteria *Proteus spp.*(Alkalophiles)

Lab Experiment:



Procedure of pH:

1-*E.coli* bacteria cultivate on nutrient broth at a temperature of 37°C for 24 hours.

2-Serial dilutions are prepared taken from the previous cultural media and works a series of dilution by placing 9 ml of distilled water in test tubes and added 1 ml to the previous tube and thus operate the other dilution.

3-Taken last diluted and divided by three clean test tubes. The first tube is measured by pH – paper(standard) PH is Neutral = 7.

4-Several drops of hydrochloric acid(HCL) are added to the second tube to make acidic media and measured by pH – paper is (acidic media).

5- Several drops of Sodium hydroxide (NaOH) are added to the second tube to make alkaline media and measured by pH – paper is(alkaline media).

6-The three tube are incubated by incubator for one hour because the generation time for these bacteria is short ranges 18-20 minutes and this is enough to grow and multiply by adequate media.

7-Taken 0.1 ml inoculums from each three tubes and is transferred onto the nutrient agar surface in the plate and is spread.

8-The three plates are incubated by incubator at a temperature of 37°C for 24 hours and read results.