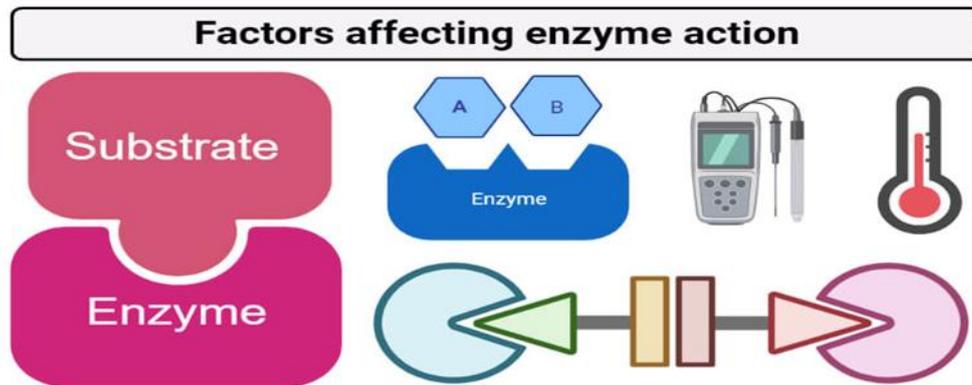


Factors affecting enzyme action and immobilized enzymes



Factors that affect enzyme action

Enzymes are biological catalysts that accelerate the rate of chemical reactions in the biological system of living beings. Like catalysts, enzymes are also affected by a number of factors that regulate enzyme action. These factors are related to the chemical nature of enzymes as enzymes are proteins, and proteins are affected by most of these factors. Some of the factors that affect enzyme action are described below:

1. Temperature

- Enzymes are thermolabile or heat-sensitive because they are proteinaceous in nature.
- Temperature affects the speed of the reaction by changing the activity of the enzyme involved.
- Like in most proteins, the rate of an enzyme action increases with the rise in temperature.
- The rate increases by two to three-fold with every 10°C rise in temperature.
- However, as the temperature becomes high, the activity of an enzyme decreases. Temperature above 60°C causes destruction and coagulation of enzymes.
- This temperature is detrimental to enzymatic reactions as the structure of the enzymes changes irreversibly.
- Some enzymes found in dry tissues, however, can endure higher temperatures like 100°C to 120°C.
- While studying the effect of increasing temperature, it can be observed that the initial velocity of the reaction steadily increases with temperature.
- However, once a particular temperature is crossed, the enzymatic activity begins to cease with less and less product being formed.

- That particular temperature or temperature range is termed as an optimal temperature of an enzyme. This temperature not easy to determine precisely because it is a somewhat vague concept, and will depend on the length of time over which the measurements are made.
- Despite that, the approximate values obtained often show a distinct correlation with the body temperatures of the organisms from which the enzyme came.
- Thus, enzymes found in mammals have optimal temperature in the range of 35°C to 45°C, but enzymes in bacteria living in hot springs may have an optimal temperature of 80°C.
- At low temperatures, the catalytic activity of the enzyme predominates, although some thermal denaturation takes place during this period.
- As the temperature reaches 0°C, inactivation of the enzyme might be observed, which is a reversible type of change and the enzyme regains its catalyzing power upon increasing the temperature to the optimum.
- The effect of temperature and heat is also observed during the storage of enzymes as the best preservation of enzyme preparations is by refrigeration or quick freezing.

2. pH (using buffer solutions)

- pH is another important parameter that affects the activity of the enzyme by changing its shape and structure.
- Like temperature, pH, or the H⁺ ion concentration of the medium where the enzyme is present bring about significant changes in the activity of such enzymes.
- The change in pH causes ionization of amino acid atoms and molecules while also changing the degree of dissociation of the substrate.
- Besides, a change in pH might also bring changes in the charges present on the enzyme, which affects the formation of the enzyme-substrate complex.
- Thus, enzymes have a particular value of pH or concentration of H⁺ ion at which the enzyme acts best.
- The activity of the enzyme, however, decreases with any change (increase or decrease) in the said pH value.
- This specific pH at which the activity of an enzyme is maximum is termed as the optimal pH for that particular enzyme.
- This pH might be specific to each enzyme and is determined by various factors like the composition and structure of the enzyme.

- Other factors that determine the optimum pH for an enzyme include the nature of the buffer system, the presence of other colloids, activators, or inhibitors, the age of the cell tissue, and the nature of the substrate.
- Change in the pH of the buffer solution used brings about changes in the activity of an enzyme, as it affects the structure and shape of the enzyme.
- The use of different buffer solutions with lower or higher pH values might affect the ionization state of the acidic (carboxyl) or basic (amine) groups.
- With the change in the ionized state of amino acid, the ionic bonds maintaining the three-dimensional structure of the enzymes are also affected.
- This might lead to a reduction in enzyme activity and even inactivation.
- pH is also found to change the structure and shape of substrates which prevents the binding of substrate to the active site of the enzyme.
- These changes might be reversible for a narrow range of pH, but if the pH change is significant, enzymes and substrate might be denatured, causing permanent loss of activity.

3. Enzyme concentration

- The effect of enzyme concentration on the activity of an enzyme can only be observed when the substrate is present in excess, causing the reaction to be independent of the substrate concentration.
- In that case, any change in the number of products formed over a particular period of time will be dependent on the enzyme concentration.
- Thus, to observe the effect of enzyme concentration, zero-order reactions are to be studied.
- In order to determine the concentration of enzyme in a system, the amount of substrate catalyzed is to be determined. This, in turn, depends on other factors like temperature and pH.
- However, to determine the relationship between the concentration of enzyme and rate of enzyme action, the substrate must be present in excess, resulting in a zero-order reaction.
- Under such conditions, enzyme action increases linearly with time, causing double the amount of products to be formed as the process is run for double time.
- The speed of the reaction or the activity of the enzyme increases with the increase in enzyme concentration as long as there are enough substrate molecules to bind to the active sites of the enzyme.

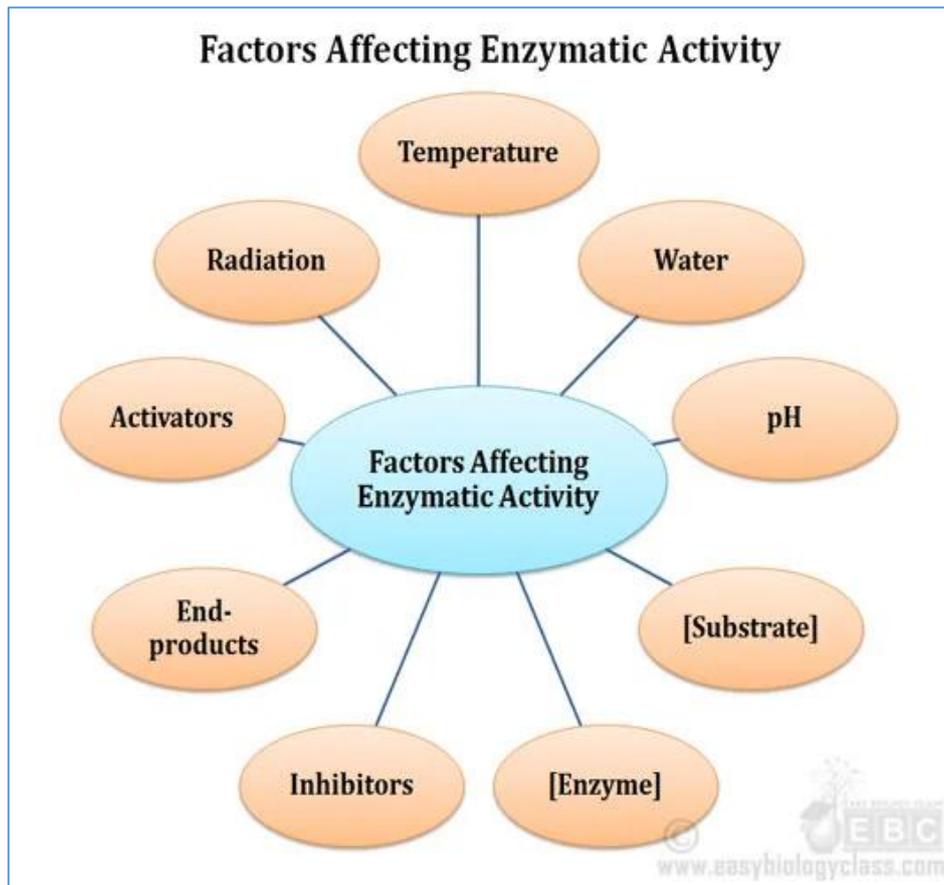
- Once all the active sites are filled, the enzyme activity doesn't increase.

4. Substrate concentration

- The effect of the increase in substrate concentration on the enzymatic action is to be determined at a constant concentration of the enzyme.
- When the concentration of the enzyme is constant, the rate of a chemical reaction or the activity of the enzyme increases with the increase in substrate concentration up to a point where it is maximum.
- After this point, the increase in substrate doesn't change the activity of the enzyme or the rate of the reaction.
- It is because, with an increase in substrate concentration, the number of substrate molecules binding to the active site of the enzyme increases.
- But once all the active sites are filled, increasing substrate concentration doesn't affect the activity of the enzyme.
- Thus, the enzyme action is the highest when all enzyme molecules are present in the form of the enzyme-substrate complex.

5. Inhibitor concentration

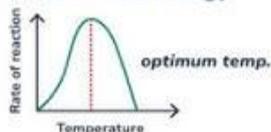
- Inhibitors are compounds that convert the enzymes into inactive substances and thus adversely affect the rate of enzymatically-catalyzed reaction is called an enzyme inhibitor, and the process involved is termed enzyme inhibition.
- These molecules might affect the activity of the enzyme by either binding to the active site or some other regions of the enzyme.
- This prevents the binding of substrate to the active site, affecting the rate of formation of the enzyme-substrate complex.
- The concentration of such inhibitors is indirectly proportional to the rate of enzyme action.
- With the increase in the concentration of inhibitors, the rate of formation of enzyme-substrate complex decreases which, in turn, decreases the concentration of products formed.
- Depending on the nature of inhibition (competitive or non-competitive), the effect of inhibitors might be reduced by changing the concentration of the substrate.
- In competitive inhibition, the rate of enzyme action can be increased by increasing the substrate concentration in the medium.
- In non-competitive inhibition, however, the rate cannot be increased even with the increase in substrate concentration.



Factors Affecting Enzyme Activity

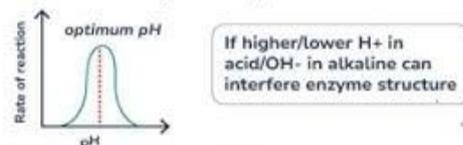
Temperature

BIG influence More heat
= More kinetic energy



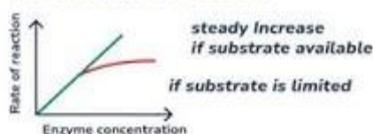
pH

Enzymes have optimum pH



Enzyme concentration

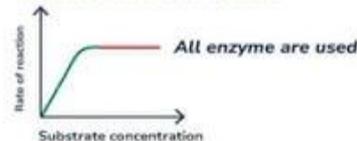
Increase enzyme concentration
= increase rate of reaction



****Until substrates amount are limited***

Substrate concentration

Increase substrate concentration
= increase rate of reaction



****Until active site of enzyme are used****