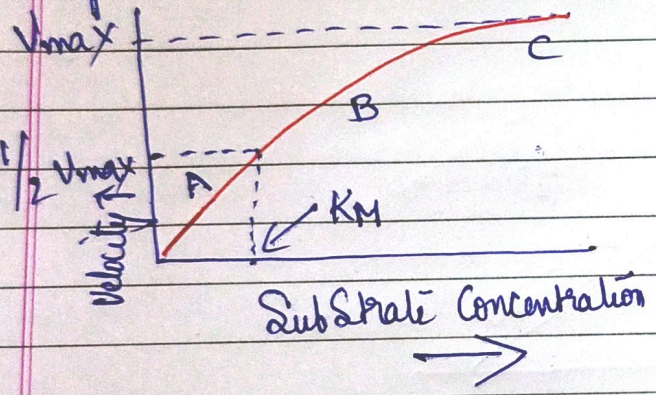
Factors Affecting enzyme activity

(1) Concentration of enzyme :-

As the concentration of the enzyme is increased, the velocity of the reaction increases proportionally. This property is used to estimate the serum enzymes for the diagnosis of disease.

(2) Concentration of Substrate :-

Increase in the Substrate Concentration gradually increases the velocity of enzyme reaction.
A rectangular hyperbola is obtained when velocity is plotted against the Substrate Concentration. The graph has 3 distinct phases A - linear
B - Curve
C - almost unchang



order of reaction :- when the velocity of the reaction is almost proportional to the Substrate Concentrations, the rate of reaction is said to be first order. when the Substrate Conc. is much greater than the velocity of reaction the rate of reaction is independent of Substrate Concentration & the reaction is said to be zero order.

Enzyme Kinetics & K_m value :-

The enzyme [E] & Substrate [S] Combine with each other to form an unstable enzyme - Substrate Complex [ES] for the formation of product (P).

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_3} E + P$$

K_1, K_2, K_3 } - Represent velocity constants for the respective reactions.

K_m - Michaelis - Menten Constant (Brig's & Haldane's Constant)

$$K_m = \frac{K_2 + K_3}{K_1}$$

After Manipulation the following Equation is obtained

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

Equation - (1)

V = Measured velocity
V_{max} = Maximum velocity.
S = Substrate Concentration
K_M = Michaelis - Menten constant.

If we assume measured velocity is half of maximum velocity ($\frac{1}{2} V_{max}$). The Equation becomes

$$\frac{1}{2} V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

$$K_m + [S] = \frac{2 V_{max}[S]}{V_{max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S].$$

Now K_M is defined as (Michaelis - Menten Constant) is defined as the substrate concentration to produce half - maximum velocity in an enzyme catalysed reaction.

K_m value is a constant & a characteristic feature of a given enzyme. It measures the strength of ES complex. A low K_m value indicates a strong affinity between enzyme & substrate where as high K_m value indicates weak affinity b/w them.

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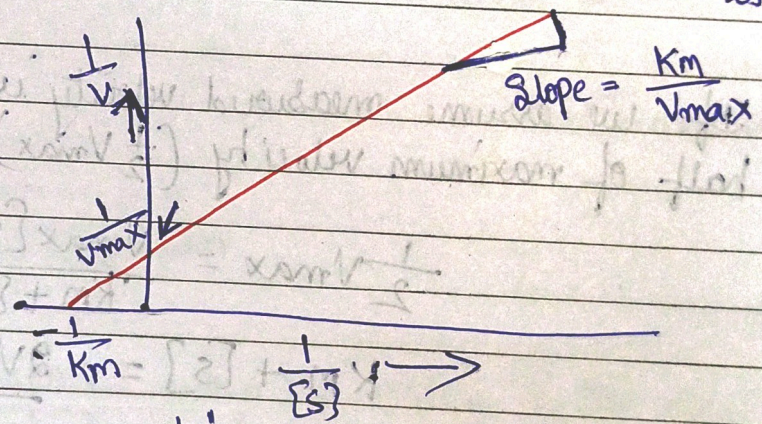
Line-weaver burk double Reciprocal plot :-

For the determination of K_m value, the substrate Saturation Curve is not accurate. By taking the reciprocals of Equation (1) a straight line graphic Representation is obtained.

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]}$$

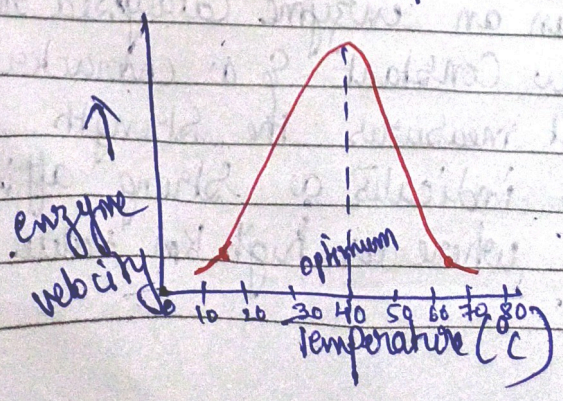
$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

This Equation is similar to $y = ax + b$, Therefore a plot is obtained for $\{1/v\}$ Reciprocal of velocity VS $\left\{\frac{1}{[S]}\right\}$ Reciprocal of substrate, this gives a straight line. Here the slope is K_m/V_{max} & y intercept is $1/V_{max}$.



Line weaver - Burk double Reciprocal plot.

(3) Effect of temperature :- velocity of an enzyme reaction increases with increase in temperature up to a maximum & then declines. A bell shaped curve is usually observed.



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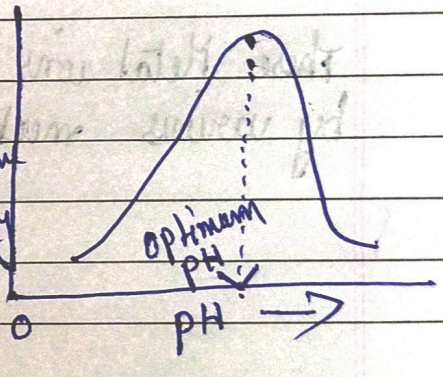
Temperature Coefficient - (Q_{10}) is defined as increase in enzyme velocity when the temperature is increased by 10°C. For a majority of enzymes, Q_{10} is 2. between 0°C & 40°C. The optimum temperature for most of the enzymes is between 35-40°C. However some enzymes are active at 100°C - This is due to their stable structure & conformation.

Generally, enzymes when exposed to a temperature above 50°C undergo denaturation (change in structure & active site).

Significance :- Foods can be preserved in refrigerators, bacterial enzyme activity is less at low temp.

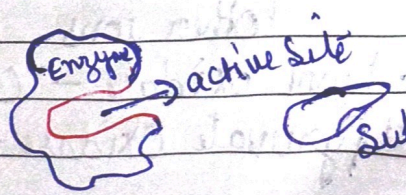
(4) Effect of pH :-

Increase in H^+ ion concentration changes the enzyme activity. Generally a bell shaped curve is obtained when pH is plotted against enzyme velocity.



Every enzyme will have an optimum pH, below & above this optimum pH the enzyme activity is lower.
Most of the enzymes show an optimum activity at neutral pH (6-8) but many exceptions are there like pepsin (1-2), acid phosphatase (10-11).

(5) Effect of product concentration :-



The accumulation of products generally decreases the enzyme velocity, this is prevented by a quick removal of products formed.

- The products combine with active site of enzyme & forms a loose complex, this complex formation inhibits enzyme activity.

(6) Effect of activators:-

Some of the enzymes need metallic ions like Mg^{+2}, Mn^{+2}, Zn^{+2}, Ca^{+2}, Co^{+2}, Ce^{2+}, Na^{+}, K^{+}, Cl^{-} for producing optimum activity.

These Metal ions acts as activators & increases enzyme velocity by various mechanisms like → Combining with Substrate,
→ formation of E.S complex,
→ direct participation in Reaction
→ Brings a Conformational change in Enzyme.

(1) Metal-activated Enzymes:-

The metal do not bind tightly with enzyme & can be easily exchanged with other ions.

Eg:- ATPase (Mg^{+2} & Ca^{+2})
Enolase (Mg^{+2})

(2) Metalloenzymes:-

These metals hold the enzymes tightly & cannot be easily exchanged with other ions.

Eg:- phenol oxidase - Copper
Pyruvate Oxidase - Manganese.

7) Effect of time :-

under ideal Conditions like optimum pH & temperature the time required for a reaction to occur is less. If there is alterations in pH & temperature, the time required for a reaction to occur will change.

8) Effect of light & Radiation :-

When enzymes are exposed to UV, β, γ & X-rays they get inactivated due to formation of peroxides.

Eg :- U.V. rays Inhibit Salivary amylase activity.