VIVEKANANDA COLLEGE
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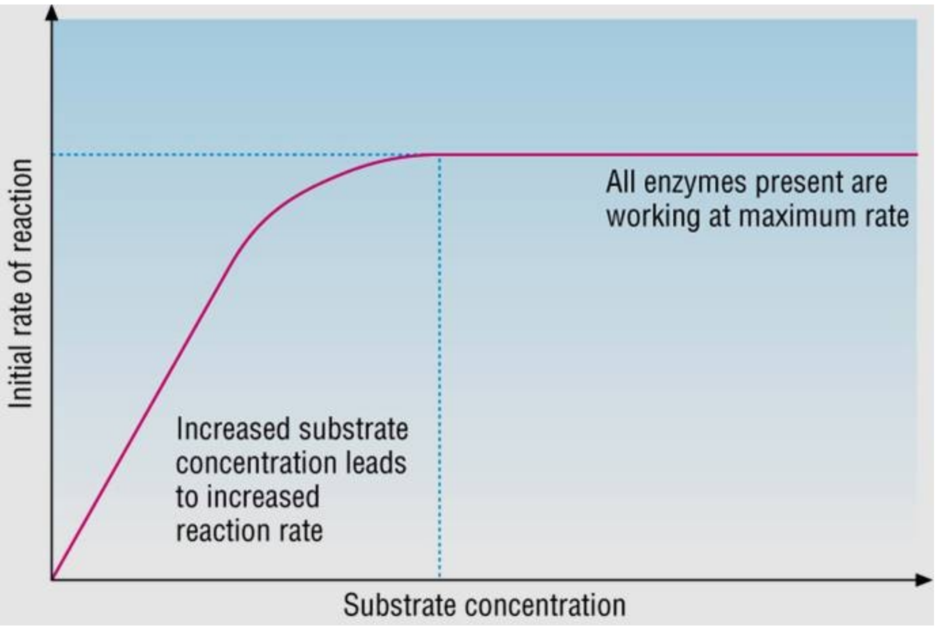
NAAC ACCREDITED ‘A’ GRADE

Topic : Enzyme Kinetics
Course Title : Enzymes
Paper : CC4
Unit : II
Semester : 2
Name of the Teacher : Dr. Kakali Roy
Name of the Department : Biochemistry

Factors affecting Enzyme Catalysed Reactions

- **Substrate Concentration**



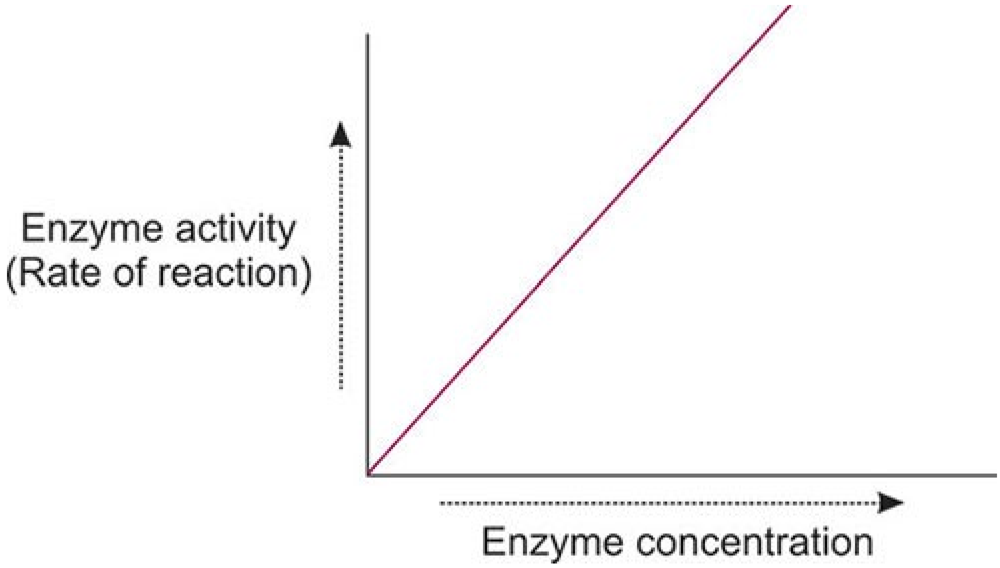
- Reaction velocity (V) is plotted against substrate concentration [S], a hyperbolic curve is obtained.

- At low substrate concentration, rate of the reaction increased with [S], i.e. $V \propto [S]$
- At high substrate concentration, velocity does not increase with increase in [S], i.e. velocity remains constant and parallel to X-axis.

- All the enzyme is saturated with the substrate.

- V is independent of [S].

❖ Enzyme Concentration



- ❖ Effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration.

- ❖ A linear curve is obtained indicating $V \propto [E]$.

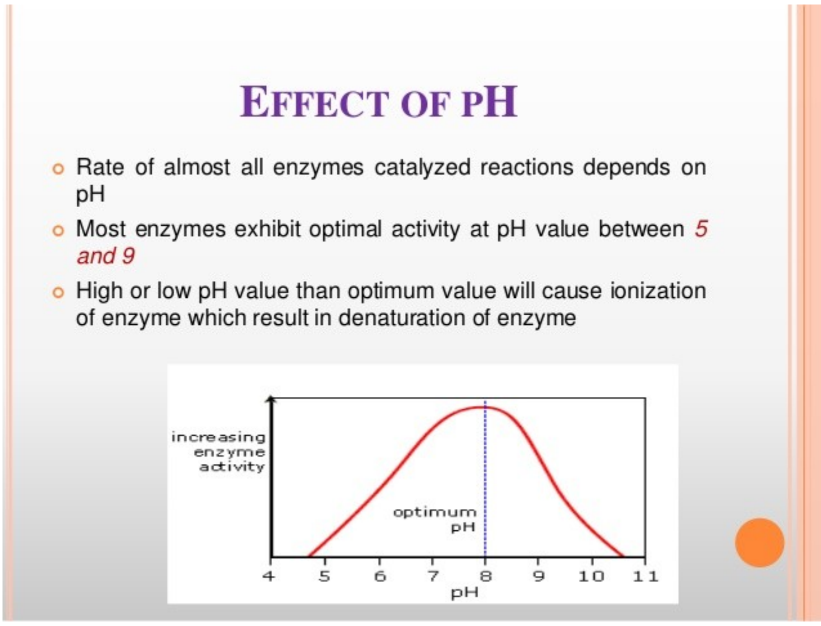
- ❖ Reaction follows 1^{st} order kinetics w.r.t enzyme concentration.

- ❖ For in vitro velocity determination, enzyme concentration is usually kept low, so the available substrate can saturate the enzyme.

➤ pH

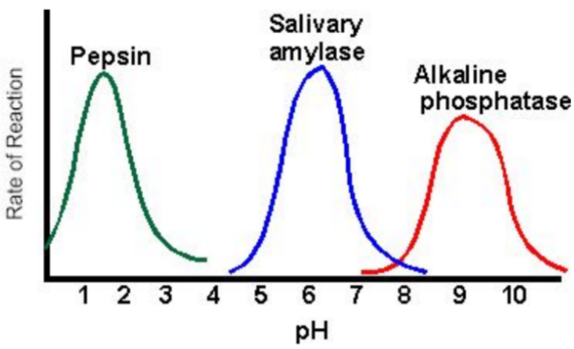
- Activity of the enzyme is influenced by the pH of the medium.

- Activity is maximum at optimum pH.

- 

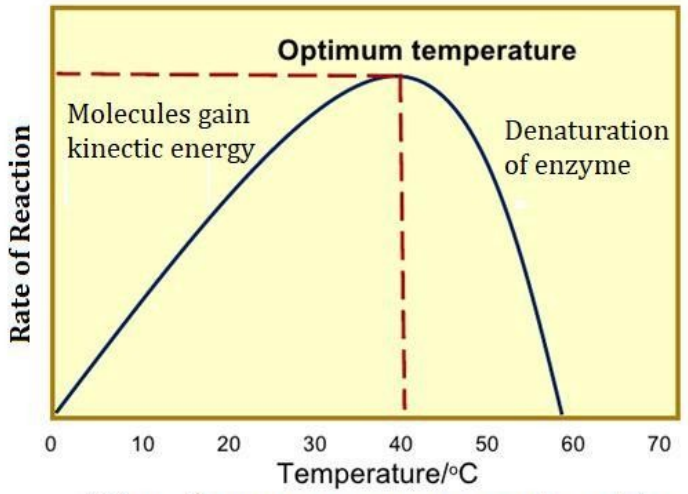
Plot of activity vs pH ------ A bell shaped curve is obtained.

Optimum pH may vary for different enzymes



- Pepsin, a proteolytic enzyme has an optimum pH as low as 1.5
  - Salivary amylase has an optimum pH around 6.0.
  - Alkaline phosphatase has optimum pH in alkaline medium (9- 10)

Temperature



Effect of temperature on enzymatic activity

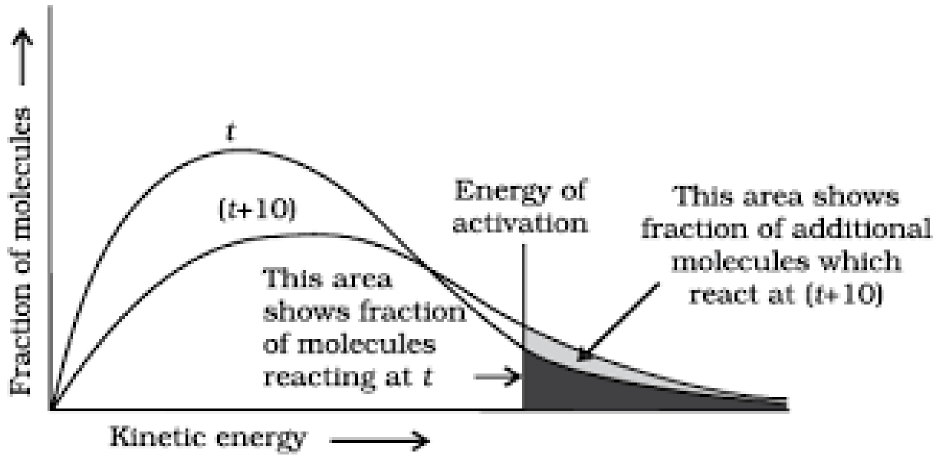
- Every enzyme shows a maximum activity at optimum temperature.
- Optimum temperature range of most of the enzyme ----- 35-40°C.
- Plot of Activity vs temperature --------- a bell shaped curve is obtained.
- The temperature coefficient (Q_{10}) i.e. rise in reaction rate for 10°rise in temperature is increased by 2 fold for most enzymatic reactions but the rate of denaturation increases several times.

❖ Temperature dependence of Rate constant

❖ Rates of reactions increase with increase in temperature of the reaction mixture.

❖ When the temperature increases, the fraction of molecules that have kinetic energies more than the activation energy of the reaction increases.

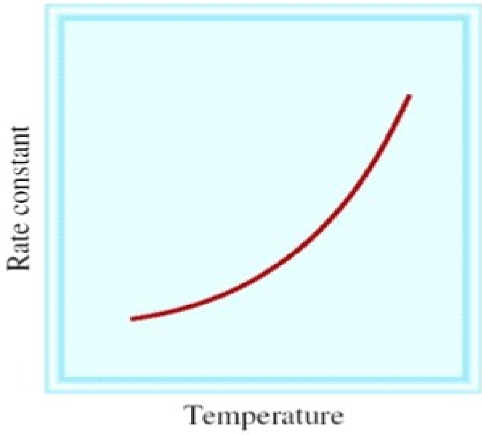
❖ The total activation energy of the reaction decreases. The rate of the reaction increases.



❖ Keeping the concentration of the reactants constant, the rate is found to be two times its initial value, when measured at a temperature 10 K greater than the initial temperature.

❖ **The exact value of the rate constant determined at various temperature is predicted by using Arrhenius equation.**

Temperature Dependence of the Rate Constant



$$k = A \cdot e^{(-E_a / RT)}$$

(Arrhenius equation)

E_a is the activation energy (J/mol)

R is the gas constant (8.314 J/K•mol)

T is the absolute temperature

A is the frequency factor

Alternate format:

$$\ln k = -\frac{E_a}{R}\frac{1}{T} + \ln A$$

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➢ Arrhenius Equation

- The Arrhenius equation is a formula for the temperature dependence of reaction rates proposed by Svante Arrhenius in 1889.
- This equation has a vast and important application in determining rate of chemical reactions and for calculation of energy of activation.

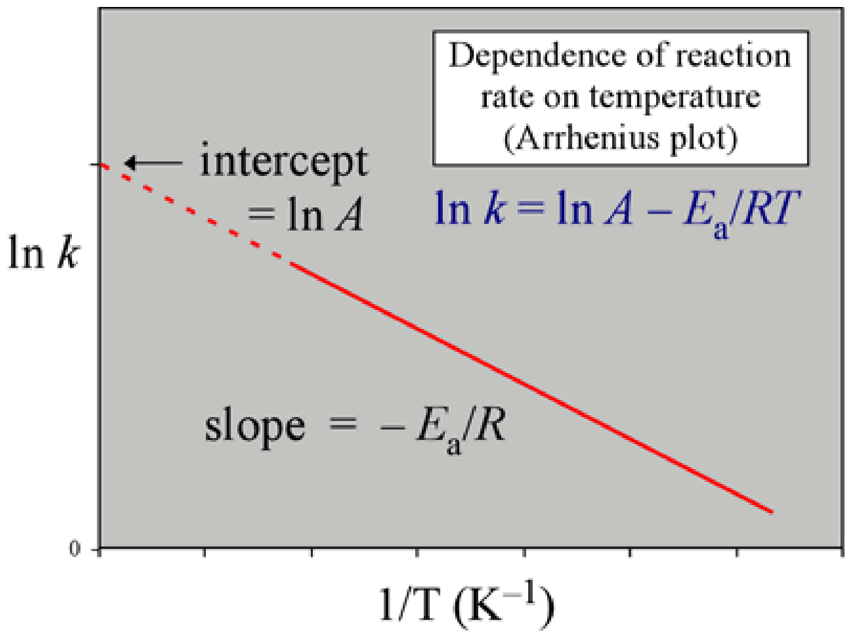
- **Arrhenius equation** : **$k = A\, e^{-Ea/RT}$**

where k = rate constant, E_a = activation energy, A = frequency factor, R = gas constant, T = temperature in Kelvin.

Taking the natural logarithm

$\ln k = \ln A - E_a / RT$

or, $\ln k = -E_a /RT + \ln A$



Plot of ln k against 1/T gives a straight line : Slope = $-E_a/R$ and intercept = ln A.

- If $\mathbf{k_1}$ and $\mathbf{k_2}$ are the rate constants measured at two different $\mathbf{T_1}$ and $\mathbf{T_2}$ temperatures respectively, then $\mathbf{E_a}$ can be determined as follows :

- Arrhenius equation for two different temperatures T_1 and T_2 are :

- $\ln k_1 = \ln A - (E_a /RT_1)$ and $\ln k_2 = \ln A - (E_a /RT_2)$

- $\ln k_2 - \ln k_1 = - (E_a /RT_2) + (E_a /RT_1)$

- **$\ln (k_2 /k_1) = E_a / R (T_2 - T_1 / T_1T_2)$**

  If R = 1.987 cals.mol^{-1} , then unit of E_a is 10^{-3} kcal.

- When E_a is a positive value, and if $T_2 > T_1$ then $k_2 > k_1$.

- **Rate constant value at higher temperature is greater than rate constant value at lower temperature**.

- High temperature and low activation energy favour larger rate constants and thus speed up the reaction.

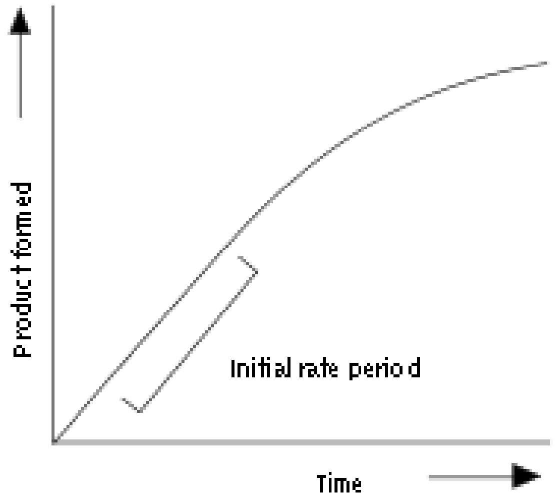
- Under such conditions, plot of ln k against 1/T gives a negative slope straight line. **From the slope of the straight line, E_a can be calculated**.

❖ Time

- The product formed is plotted against time gives initially a linear curve.

- Initially substrate concentration high → reaction velocity maximum → product formation directly proportional to time, i.e. $P \propto t$.

- Reaction continued for a longer time → high amount of substrate consumed → reaction velocity decreases → product formation not proportional to the time of the reaction.



Reference :

1. Biochemistry 3rd Edition, Voet and Voet, John Wiley & Sons, INC.
2. www.toppr.com>guides>chemistry>chemical-kinetics
3. Biochemistry, 2nd Edition, Lehninger, Kalyani Publishers
4. www.google.com