CHARACTERTISTICS OF ENZYMES

Certain characteristics of enzymes are different from inorganic catalysts. Some of these are:

A.Catalytic Power

Enzymes are the most efficient catalysts known. They can increase the rate of a reaction by a factor of up to 10^{20}over uncatalyzed reaction. However, non-enzymatic catalysts enhance the rate of reaction by factors of 10^2 to 10^6 only.

Catalysts are effective in small amounts.

When analysing the catalytic power of an enzyme, we should know the amount of starting material or substrate that is converted to product in a unit time by a given quantity of the enzyme. This quantity is called the turnover number and is defined as the number of moles of substrate converted into product per minute by one mole of enzyme.

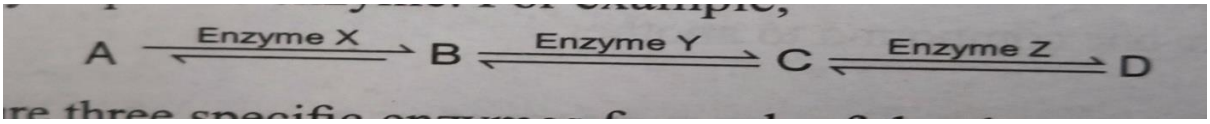
The turnover numbers of enzymes vary greatly ranging from one hundred to over three million.

Catalysts ordinarily have no effect on the equilibrium of a reversible chemical reaction. They merely speed up the reaction until it reaches equilibrium. Enzymes act in a similar way, they hasten the process in either direction.

Catalysts are usually unchanged in the reaction: This property of ideal catalysis does not hold true by enzymes. As proteins are easily inactivated or denatured by high temperature or very alkaline or acidic conditions. So, enzymes work under optimum conditions of temperature and pH.

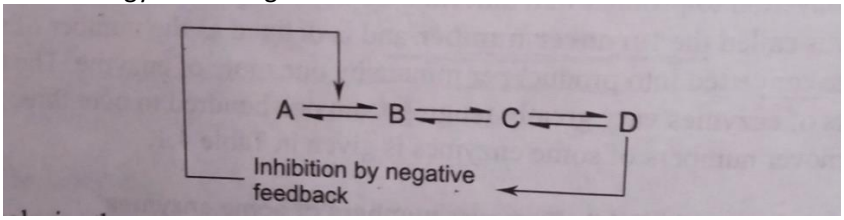
B.Control of Enzyme Action

Biochemical pathways usually involve number of linked reactions. each reaction is catalyzed by a specific enzyme for example



In this there are 3 specific enzyme for each of the 3 steps involved in the conversion of A to D. in some instances accumulation of one of the product formed near the end of the chain inhibit the action of an enzyme in one of the earlier reactions. in above example , the presence of high concentration of the product D may slow down the rate at which enzyme X convert A to B. this is called negative feedback in inhibition. negative feedback ensures that reactants are used efficiently and prevents the excess manufacture of end products. Thus, control is exercised on the pathway of above reactions. such a control of enzyme action helps to maintain stable environment in living organisms.

pathways can be shut down if an Organism has no immediate need for their products, which saves energy for the organism.



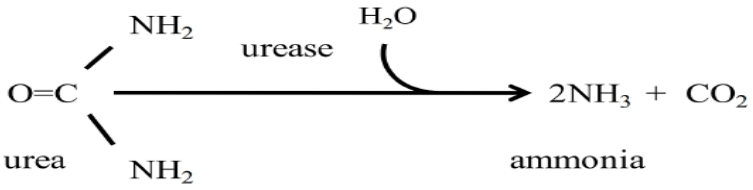
In glycolysis, 3 reactions are control points. the first is the conversion by glucose to glucose-6-phosphate, which is inhibited by glucose-6-phosphate itself. The second is the production of fructose-1,6-biphosphate, inhibited by ATP and lastly the reaction of phosphoenolpyruvate to pyruvate is also inhibited by ATP.

It is frequently observe that control is exercised near the start and at the end of a pathway as well as add points involving the key intermediates as exemplified above .

C. Enzyme Specificity

Enzymes are highly specific in their reactivities. They catalyse only one reaction or a group of closely related reactions. An enzyme may be specific with regard to the type of reaction it catalyses or with regard the kind of substrate on which it acts. that means some enzymes are reaction specific and others possess substrate specificity. For example, hydrolases carry out hydrolysis reactions and oxidase catalyse only oxidations. A carbohydrase will attack only on carbohydrates and nucleases attack on nucleic acids. Maltase catalyses the hydrolysis of the disaccharide, maltose.

Urease, however, is a unique enzyme. It catalyses only the hydrolysis of urea. It has no effect on other compounds, even closely related ones such as amides. Such absolute specificity is rather rare among enzymes characterised to date.



Among reaction specific enzymes group specificity or substrate specificity is observed side by side. For example, α-glucosidases will hydrolyse all compounds containing an α-linked glucose. Invertase catalyses both hydrolysis of sucrose and of raffinose which have same linkage, i.e, the enzyme is β-fructofuranoside. On the other hand lipases hydrolyse fat molecules irrespective of the nature of fatty acids present in the fat. Peptidase, however, are specific with regard to a particular linkage, i.e., the peptide bond but they also require a special grouping in its vicinity. carboxypeptidase for example please only those terminal amino acid in a peptide chain having a free carboxyl group. aminopeptidase on the other hand cleaves N-terminal amino acids i.e., and amino acid having free amino group in its vicinity

Substrate specificity of peptidases are as follows :

1.Pepsin
It attacks at the amino positions of aromatic amino acids, phenylalanine, tyrosine and tryptophan. it does not attack ester bond.

2.Chymotrypsin
It attacks on the carboxyl end of the amino acids. it also hydrolyses esters.

3.Trypsin
it splits only those peptide bonds on the carboxyl sides of basic amino acids, lysine and arginine.

4.Thrombin
it is even more specific. it cleaves only those bonds between arginine and glycine. it has no effect on the hydrolysis of other peptide linkage. it would not even attack the bonds between glycine and arginine.

In an oxidation-reduction reaction, we observe enzyme specificity with regard to both the reagents i.e., both the substance oxidised and that reduced. Certain dehydrogenases will transfer hydrogen from substrate for which they are specific to coenzyme-I and not coenzyme-II. Some other dehydrogenases are specific to coenzyme-II and will not transfer hydrogen to coenzyme-I.

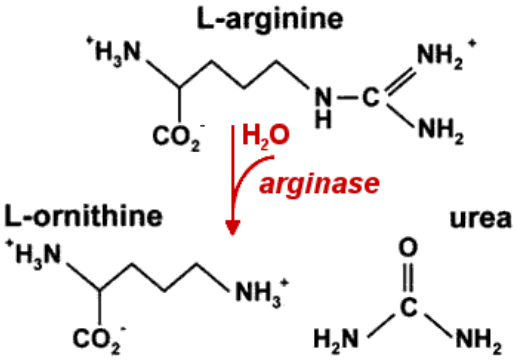
Enzyme specificity is very important in chemical reactions taking place in the cell. it ensures that proper reaction occurs in the proper place at the proper times. enzyme specificity, therefore plays a crucial role in the metabolism of the cell.

enzymes permit reactions to take place in the living organisms which otherwise would not take place at an appreciable rate at ordinary temperature and may thereby exert directive effect on the metabolism of the cell. for example by accelerating certain pathways to a greater extent than the other they may virtually direct the mechanism along one of the possible routes.

D.Stereospecificity

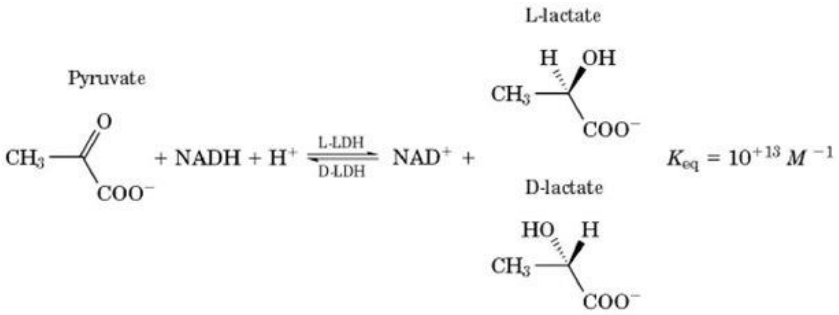
Many enzymes show stereospecificity i.e., some enzymes act on only one pair of optical isomers. In addition some enzymes are involved in asymmetric syntheses producing only one of the two possible optical isomers. The following examples show stereospecificity among enzymes:

(i)Arginase catalyses the hydrolysis of L-arginine to ornithine and urea.

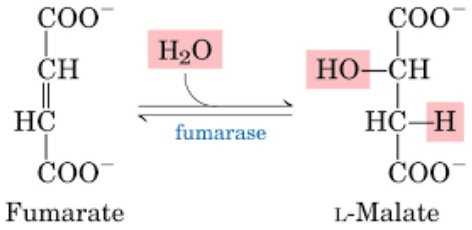


Arginase has, however, no effect on the rate of hydrolysis of D-Arginine.

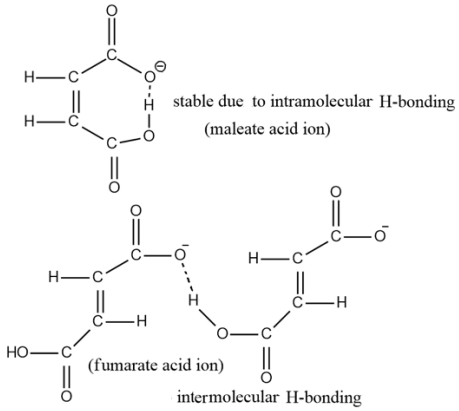
(ii) Pyruvic acid is converted to lactic acid by two different enzymes, each producing only one of the optical isomers.



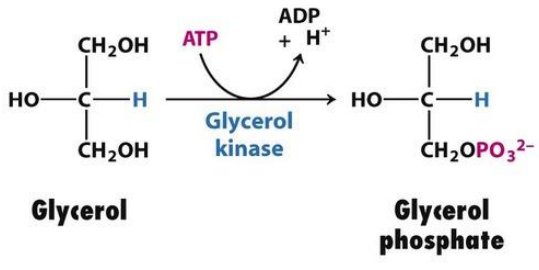
(iii)Fumarase catalyses addition of water to fumarate to give malate whereas maleate remains unaffected.



$\Delta G'^{\circ} = -3.8$ kJ/mol

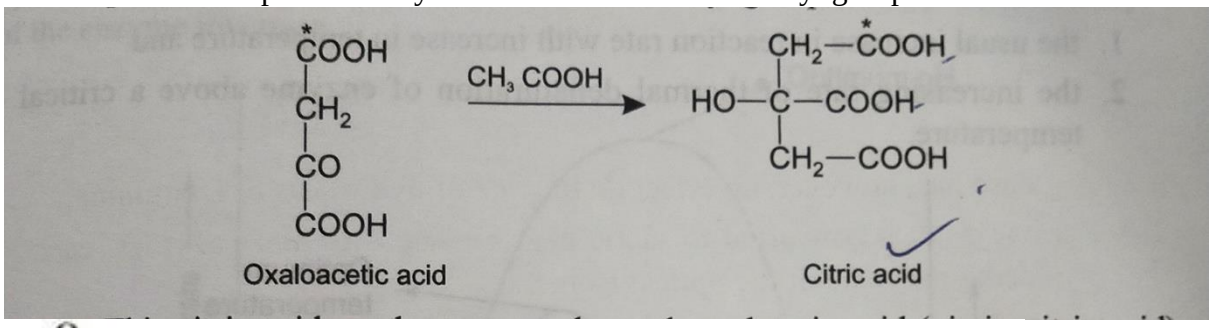


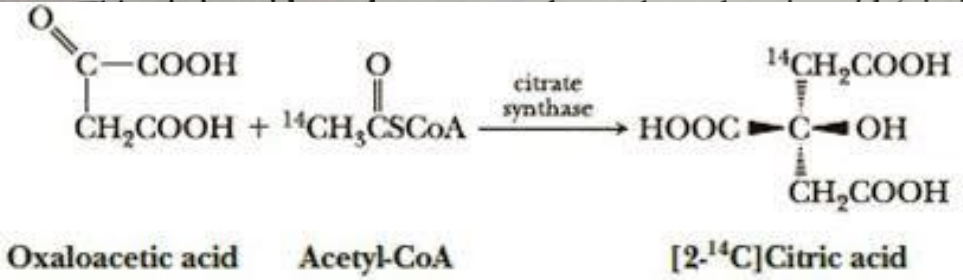
(iv) Glycerol is converted to L-phosphoglycerol by the enzyme glycerokinase phosphate group comes from ATP molecule.



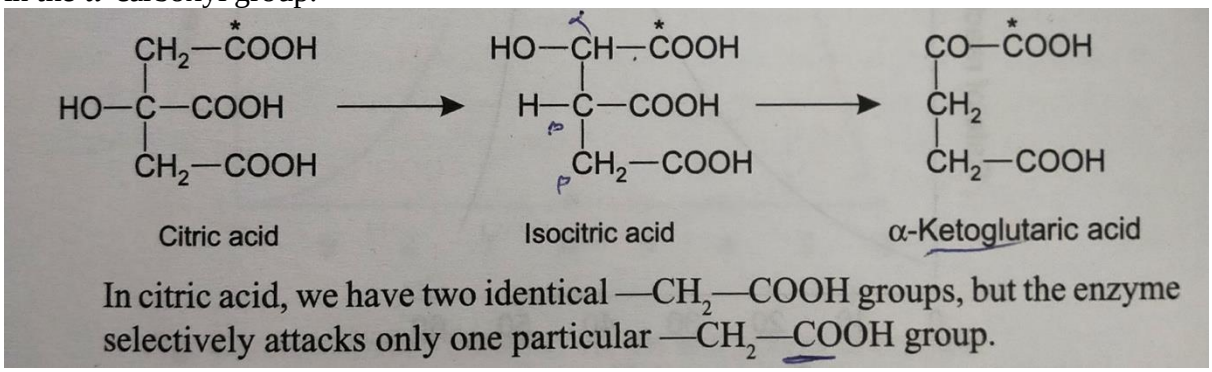
(v)During citric acid metabolism, a series of enzymatic reactions occurs in which we observe the selective action of an enzyme only on of the two chemically identical groups in a compound. For example, Oxaloacetic acid is converted to citric acid by the condensing

enzyme. If the carboxyl group adjacent to the $-CH_2$ group is labelled, the citric acid obtained will have a labelled position only in one of its terminal carboxyl groups.





This citric acid can be converted toα-ketoglutaric acid (via isocitric acid) which contains C^{16} in the α-carboxyl group.



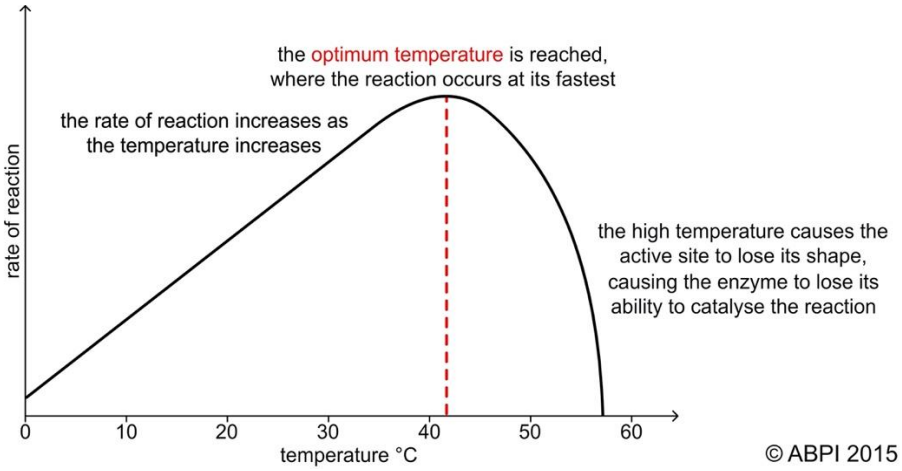
Factors Influencing enzyme activity

1.Effect of temperature on enzyme activity

The catalytic properties of enzymes are dependent on two features:
1.That the enzyme is able to form an intermediate complex with the substrate and
2.that the protein part of the molecule is preserved in its native state.
Factors which prevent either of these conditions will destroy catalysis. Thus all substance or condition which cause denaturation of enzyme will inevitably destroyed its catalytic activity.

Heat supplies kinetic energy to reactive molecules causing them to move more rapidly. the chances of molecular collision taking place are thus increased at higher temperature, so it is more likely that the enzyme substrate complex will be formed. therefore increase in temperature increase the rate of enzyme catalysis reaction. as we increase the temperature heat energy also increases the vibration of atoms which make up the enzyme molecules. if the vibration becomes too violent, chemical bond in the enzyme break and the 3 dimensional structure is lost. therefore above a certain temperature denaturation will take place and the enzyme loses activity.

enzyme catalysed reactions often appear to have optimum temperature at which the reaction proceeds most rapidly. if the temperature is raised beyond this point reaction rate decreases due to denaturation of the enzyme. this temperature is not necessarily that at which enzyme is most stable it is the resultant of 2 contrary process.
1.the usual increase in the rate of reaction is increasing temperature and
2.the increasing rate of thermal denaturation of enzyme above a critical temperature.



the rate of most enzymatic reactions approximately doubles for every 10°Crise in temperature. The term temperature coefficient (Q_{10}) is used to explain the effect of a 10°C rise in temperature on the rate of a chemical reaction

Temperature Coefficient (Q_{10})

The amount the rate of reaction increases when the temperature is raised by 10°C is known at the **temperature coefficient (Q_{10})**

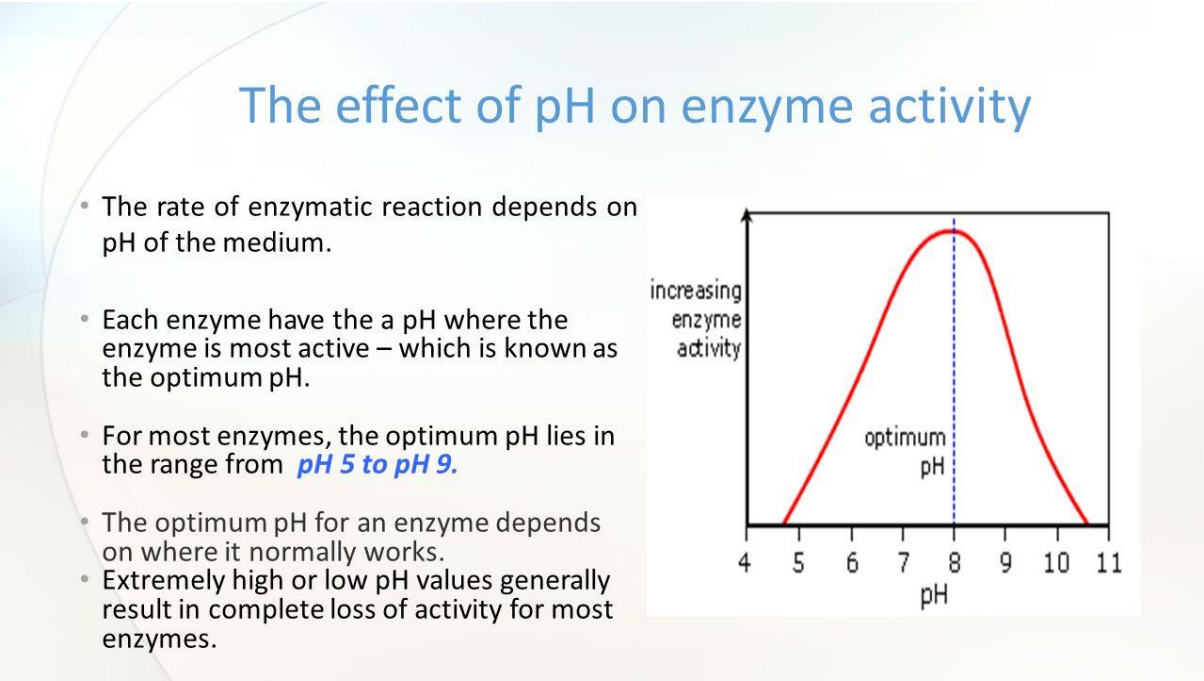
Equation:

$$Q_{10} = \frac{\text{rate of reaction at } (T + 10)^{\circ}C}{\text{rate of reaction at } T\ ^{\circ}C}$$

If Q_{10} is 2, the rate of reaction doubles for every 10^{o} rise in temperature

Optimum temperature for enzymes in the human body is 37°Celsius , the Q_{10} for enzymes catalysed reaction is 2.
Although most enzymes are inactivated at temperature above 55°C, some are quite stable and retain activity at much higher temperature example enzymes of various species of bacteria inhabiting Hot Springs are active at temperature 85°C. some enzyme such as ribosomes loses activity on heating but quickly regain it on cooling indicating that there unfolded polypeptide chain quickly reverts back to natural confirmation.

2.Effect of pH on enzyme activity
The symbol pH refers to the concentration of hydrogen ion in solution. the activity of enzyme varies the pH of the medium. most enzyme have a characteristic pH at which their activity is maximum. above or below this pH the activity declines. when we plot activity versus PH most enzyme yield Bell shaped curve with a more or less sharply defined maximum. that means enzymes have maximum activity at optimum pH.



even small changes in pH can have a great effect on enzyme activity. small changes in pH means relatively large changes in hydrogen ion concentration. A change of 1 on the pH scale involves a tenfold increase or decrease in the hydrogen ion concentration, while a change in pH of 2 represents a hundredfold change in the hydrogen ion concentration. the concentration of hydrogen ion affects the stability of the electrovalent bonds which help to maintain that tertiary structure of protein molecule. Extremes of pH causes bonds to break resulting in enzyme denaturation. the affinity of an enzyme for its substrate may be altered by variations in pH. changes in hydrogen ion concentration can alter the ionization of the amino side chains at the active centres of enzymes. Ionization of the substrate molecules can also be affected. the formation of enzyme substrate complex depends on the active centres and substrate molecules having opposite electrostatic charges. if the charges are altered by changes in pH some enzymes failed to function.

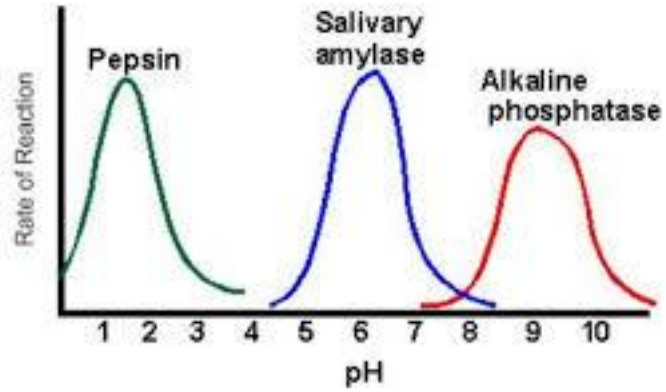
The optimum pH of an enzyme is not necessary identical with the pH of its normal intra cellular surroundings.

Optimum pH for some enzymes

Enzyme	Optimum pH
Lipase(pancreas)	8
Lipase(Stomach)	6-5
Lipase(Castor oil)	6.7
Pepsin	1.5-1.6
Trypsin	7.8-8.7
Urease	7
Invertase	6.5
Maltase	6.1-6.8
Amylase(pancreas)	6.7-7
Amylase	6.6-5.2

it is clear from the table that for every enzyme there is an optimum pH at which the reaction is catalyses proceeds most rapidly. most enzymes work within a pH range of 5 to 9 and catalyse reactions most efficiently at pH 7. there are however some exceptions for example

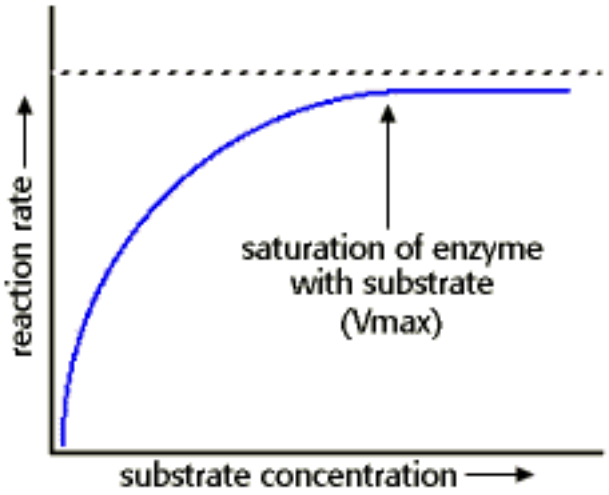
pepsin and renin secreted in the mammalian stomach work best at pH 1.5 -2.5 .alkaline phosphatase in the kidneys has an optimum pH of 10.



3.Effect of Substrate Concentration

If the concentration of the enzyme is kept constant and the substrate concentration is varied we get the reaction profile as shown.

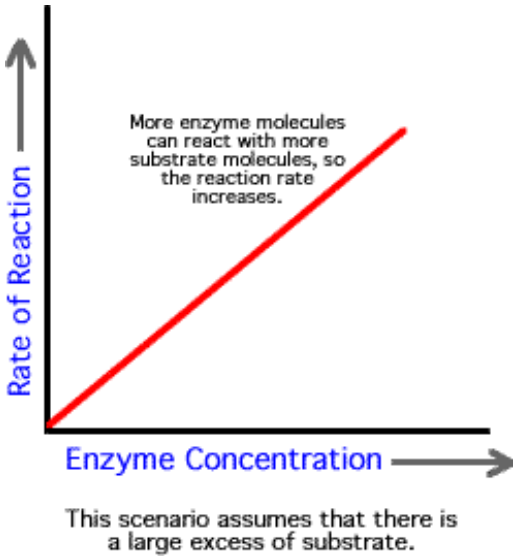
at low concentration of the substrate there is a linear relationship between the reaction rate and the substrate concentration i.e., reaction rate increases with increase in substrate concentration.
in these conditions the ratio of the enzyme to substrate molecules is high. some active sites are always free for the substrate molecules to bind with the enzyme. with increasing substrate concentration a point its reached when a further increase in substrate concentration does not cause the reaction to go any faster i.e., the reaction velocity remains unaffected. the enzyme to substrate ratios then lower and there are more substrate molecule present then the free active centre with which to bind. adding more substrate will not make the reaction go quickly. the third phase comes when the substrate concentration becomes very high the enzyme activity is inhibited by high concentration of substrate and the reaction rate declines.



4.Effect of Enzyme Concentration

Enzymes catalyse reactions rapidly at very low enzyme concentrations. if the substrate concentration is maintained constant, the reaction rate will increase as concentration of

enzyme is increased. This is because enzyme molecules form complexes with substrates only very briefly. the products of the reaction are quickly released and the enzyme is then available for further activity this relationship holds good over a wide range of enzyme concentration.



The rate at which enzymes used substrate is described as the turnover number.
for some enzymes the turnover number is very high. A molecule of catalase for example can break down 60,000 molecules of hydrogen peroxide into water and oxygen every second.
the larger the number of enzyme molecules present the greater the amount of substrate is used in a given period of time provided there is an excess substrate available.