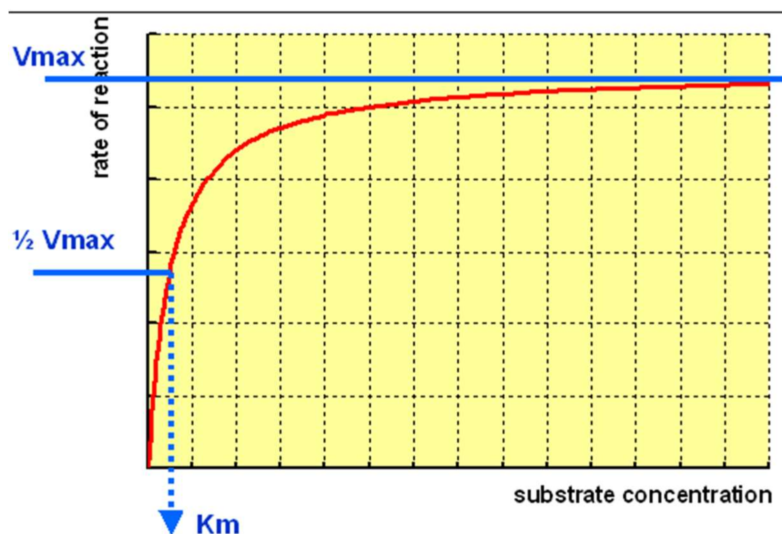
Factors Affecting the Rate of Enzyme Reactions:

Velocity or rate of enzymatic reaction is assessed by the rate of change in concentration of substrate or product at a given time duration. Various factors which affect the activity of enzymes include:

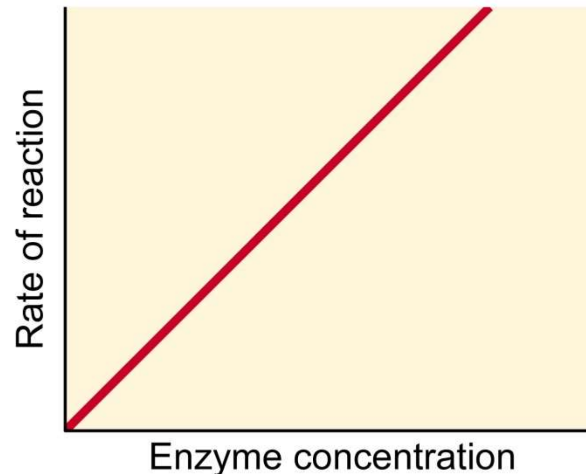
1. **Effect of substrate Concentration:**

Reaction velocity of an enzymatic process increases with constant enzyme concentration and increase of substrate concentration. The velocity (V) is expressed in micromoles of substrate converted per minute. As the substrate concentration increases, the rate of the reaction increases because more substrate molecules can collide with active sites, so more enzyme-substrate complexes form. At higher concentrations the enzyme molecules become saturated with substrate, and there are few free active sites, so adding more substrate doesn't make much difference (though it will increase the rate of E-S collisions). The maximum rate at infinite substrate concentration is called V_{max}, and the substrate concentration that gives a rate of half V_{max} is called K_m. These quantities are useful for characterising an enzyme. A good enzyme has a high V_{max} is a low K_m.



2. Effect of enzyme Concentration:

As the enzyme concentration increases the rate of the reaction also increases, because there are more enzyme molecules (and so more active sites), available to catalyse the reaction therefore more enzyme-substrate complexes form. It is worthy of note that the enzymes are rarely saturated with substrates under physiological conditions.

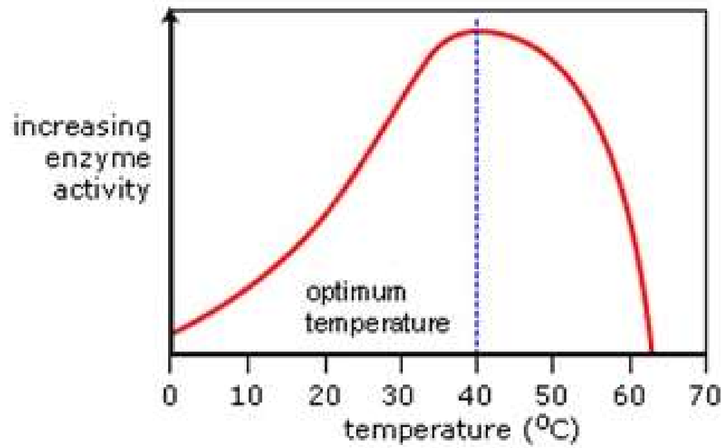


3. Effect of temperature:

Enzymes have an optimum temperature at which they work fastest. For mammalian enzymes this is about 37°C, but there are enzymes that work best at very different temperatures, e.g. enzymes from the arctic snow flea work at -10°C, and enzymes from thermophilic bacteria work at 90°C. Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more kinetic energy and so collides more often and also because more molecules have sufficient energy to overcome the activation energy.

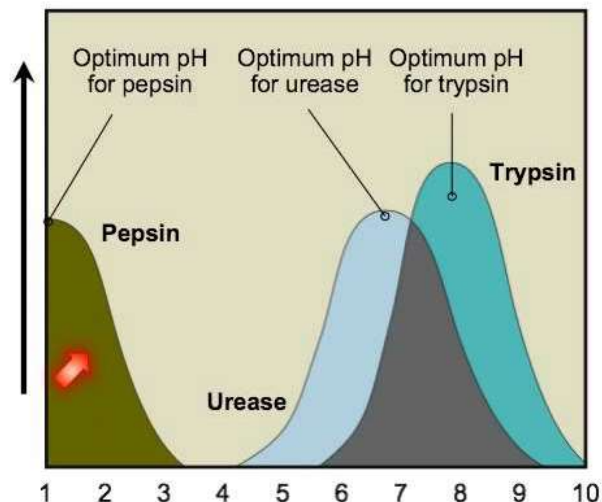
Above the optimum temperature the rate decreases as more of the enzyme molecules denature. The thermal energy breaks the hydrogen bonds holding the secondary and tertiary structure of the enzyme together, so the enzyme loses its

shape and becomes a random coil and the substrate can no longer fit into the active site.



4. Effect of pH:

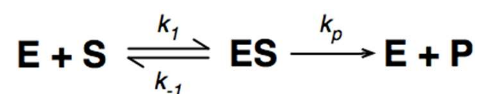
Like temperature, all enzymes have an optimum pH, at which the enzymatic activity will be at maximum. Many enzymes are most efficient in the region of pH 7-8 (normal body pH), which is the pH of the cell. Outside this range, enzyme activity drops off very rapidly. Reduction in efficiency caused by changes in the pH is due to changes in the degree of ionization of the substrate and enzyme. Highly acidic or alkaline conditions bring about a denaturation and subsequent loss of enzymatic activity. Some exceptions such as pepsin (with optimum pH 1-2), trypsin (with optimum pH about 7.5-8.5), alkaline phosphatase (with optimum pH 9-10) and acid phosphatase (with optimum pH 4-5) are even noticed.



Enzyme Kinetics:

As mentioned before, enzymes are protein catalysts that, like all catalysts, speed up the rate of a chemical reaction without being used up in the process. They achieve their effect by temporarily binding to the substrate and, in doing so, lowering the activation energy needed to convert it to a product.

In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics. The critical feature in their treatment is that a specific ES complex is a necessary intermediate in catalysis. The model proposed, which is the simplest one that accounts for the kinetic properties of many enzymes, is



An enzyme E combines with substrate S to form an ES complex, with a rate constant k_1. The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k_{-1} or it can proceed to form product P with a rate constant k_2.

According to the model above the Michaelis-Menten equation describes how reaction velocity varies with substrate concentration is written as:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Where V_0 is initial reaction velocity, V_{max} is maximum velocity, [S] is substrate concentration and K_m is Michaelis constant = $k_{-1} + k_2 / k_1$

This equation accounts for the enzyme kinetic. At very low substrate concentration, when [S] is much less than K_m, $V_0 = (V_{max}/K_m)[S]$; that is, the rate is directly proportional to the substrate concentration, Figure 3. At high substrate concentration, when [S] is much greater than K_m, $V_0 = V_{max}$; that is, the rate is maximal, independent of substrate concentration, Figure 4.

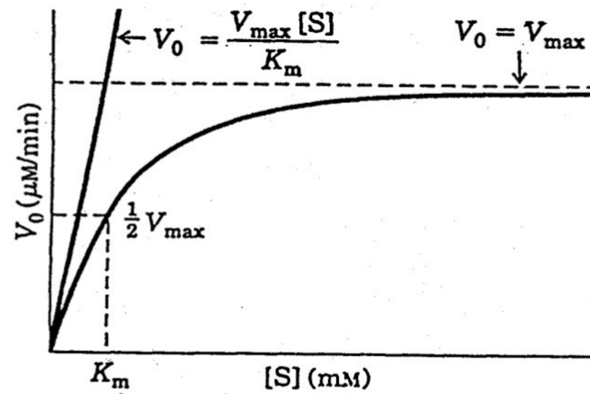


Figure 4: Michaelis-Menten curve for enzyme reaction.

Characteristics of K_m:

K_m (the Michaelis constant) is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate. K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to ½ V_{max}. K_m does not vary with the concentration of enzyme.

Small K_m: A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme, Figure 5.

Large K_m: A numerically large (high) K_m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme, Figure 5.