3.8 Factors Affecting Enzymes

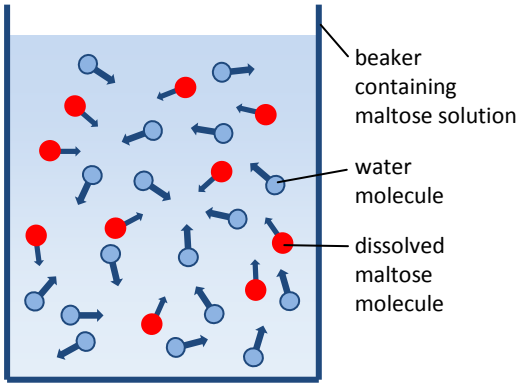
The effects of temperature, pH, concentration and inhibition on enzyme action

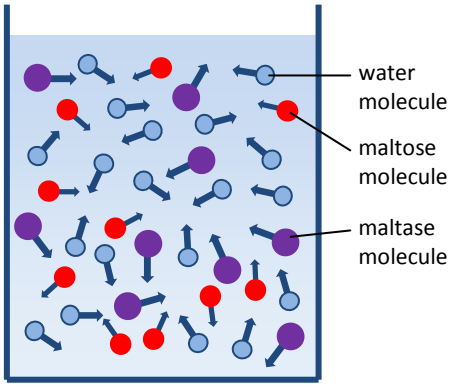
Enzymes do not function as simply as was explained in 3.8 Enzyme Action because there are a wide range of factors which affect their action. The main ones which you need to know about are discussed here.

Enzymes and temperature

Basic chemistry knowledge should remind you that molecules in a gas or liquid are constantly moving around, because they have natural *kinetic energy*. They continually collide with each other, which can initiate a chemical reaction. When the fluid is heated, their kinetic energy levels increase, and so they speed up, and the number of collisions become more frequent and with more force per collision.

An enzyme can only catalyse a reaction if the *substrate* collides with enough force into the *active site* of the enzyme, so that an **enzyme-substrate complex** is formed. It is the *random movements* of molecules which enable these collisions.



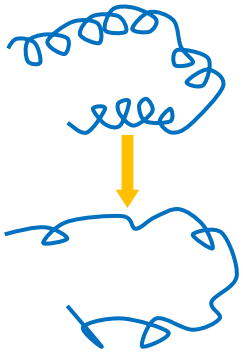


If we look at the beaker on the left, which contains only water and dissolved maltose, we know that the molecules will all continually and randomly move around and collide with each other – this is known as **Brownian motion**.

But when we add the maltose enzyme molecules (**maltase**), we enable reactions to take place more easily. When a water molecule and a maltose molecule both collide with a maltase molecule, the reaction incurs, and the product (glucose) will be produced. For details on the process of substrate-to-product, see 3.8 Enzyme Action.

So knowing that the higher the temperature, the more collisions and the more the reactions, and also knowing that the presence of enzymes will increase the number of reactions, we can assume that an increase in temperature will increase **reaction rate**?

Rate of reaction - the speed of a reaction, or how fast one reaction takes place



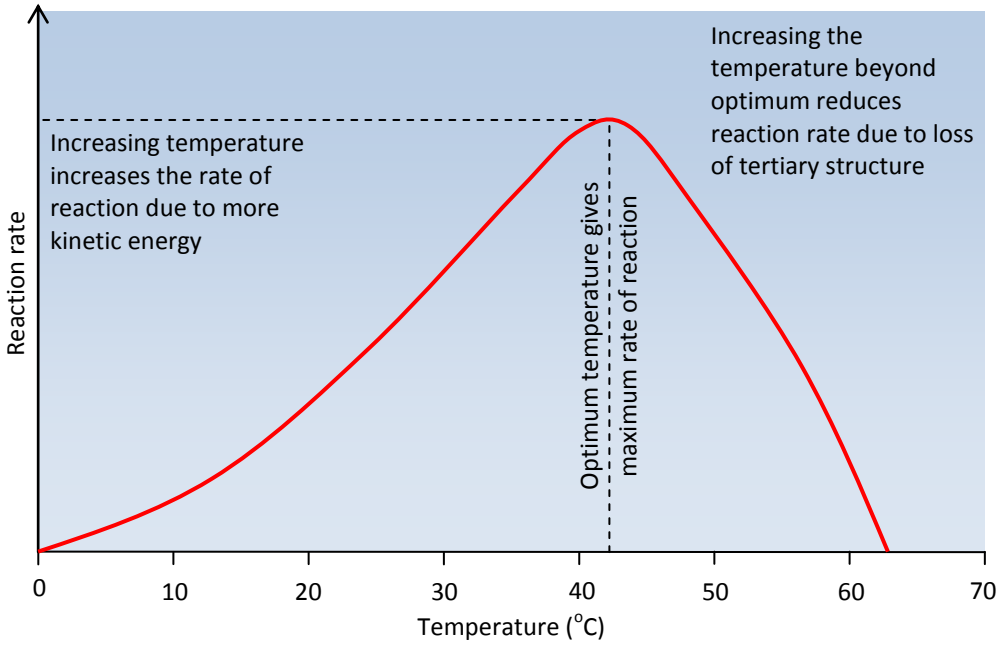
No – this is true up until a certain point, but too high temperatures can cause problems with enzymes. This is because not only do higher temperatures increase the speed of the molecules movement, but they also make them vibrate, which puts strain on the bonds holding them in place. Weak bonds, like the hydrogen and ionic bonds in enzymes are broken when they vibrate at high temperatures. The problem is that these are the bonds responsible for maintaining an enzyme's tertiary structure, and more importantly, its active site. As more and more bonds break, the enzyme loses its tertiary structure and the proteins unravel, so the enzyme can no longer function – this of course, reduces reaction rate. This is called **denaturation**.

However, it is important to note that this does *not* affect the primary structure of the proteins which make up the enzyme. The peptide bonds which give it the primary structure are *covalent*, and so are not broken very easily.

Because increasing temperature improves the rate of reaction up to a certain point, but increasing it too much will decrease the reaction rate, we say that enzymes have an **optimum temperature** for operation (that is, the perfect temperature where they are at their highest efficiency).

Denaturation - when an enzyme is heated too much it loses its tertiary structure as the hydrogen and ionic bonds holding it together are broken, so the active site is destroyed – the enzyme is denatured

The optimum temperature for the work of an enzyme will depend upon the enzyme, because they all have different functions, they all work best in different environments. The vast majority of enzymes have an optimum temperature of between 40°C and 50°C, but those enzymes which must be *heat-resistant*, such as in organisms where having such enzymes would be useless. The high temperatures of their environment would not allow enzymes to cope.

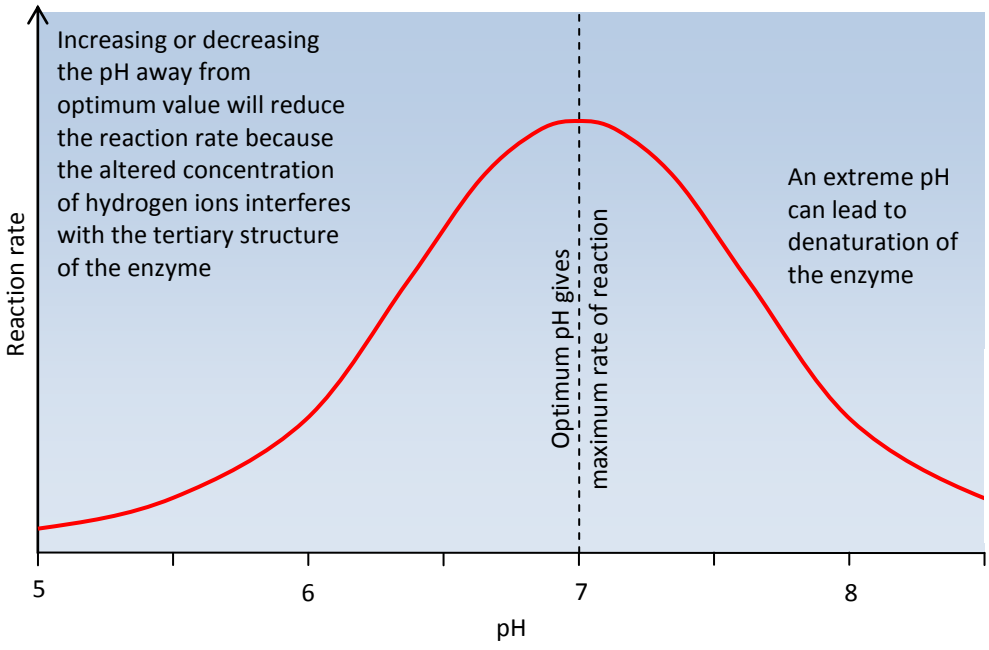


Enzymes and pH

pH is a measure of the concentration of H^+ ions, with a scale of numerical values from 1 to 14, where pH7 is **neutral**, anything below is **acidic**, and anything above is **alkaline** (or **basic**). An acid is known as a **proton donor** (because hydrogen ions are protons) and a base a **proton acceptor**.

Due to the positive charge of the hydrogen ion, it will be attracted to negatively-charged ions, molecules or parts of molecules, and repel positively-charged ions, molecules or parts of molecules. The bonds in enzymes which hold its tertiary structure and determine the shape of the active site are hydrogen and ionic bonds which both form according to *electronegativity*. It is down to the different charges each amino acid groups have in the polypeptide chains.

Obviously, the charge of these hydrogen ions therefore means that they are going to interfere with the bonds holding an enzyme's structure. So increasing or decreasing the pH will affect the concentration of the H^+ ions and in turn, alter the enzyme's tertiary structure. Again, this changes the shape of the active site, and also the rate of reaction.

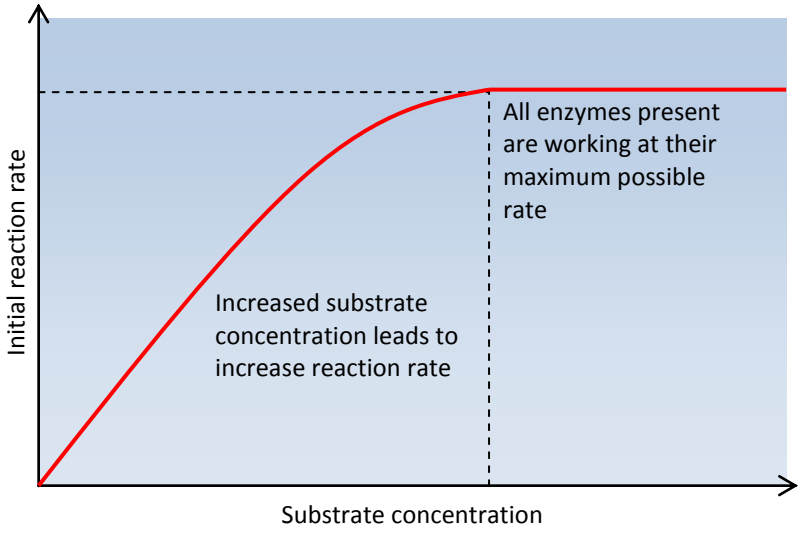


The optimum pH varies between individual types of enzyme. For many of them, however, it around neutral pH – pH7. Because enzymes normally work in a very narrow pH range environment, a sudden drop or increase in pH will cause the rate of reaction to decrease dramatically.

A minor change in the pH will not, however, cause an enzyme to denature. The bonds will still be disrupted, but not always to an extreme effect, they can be reformed. However, extreme pH changes may lead to denaturation.

Enzymes and concentration

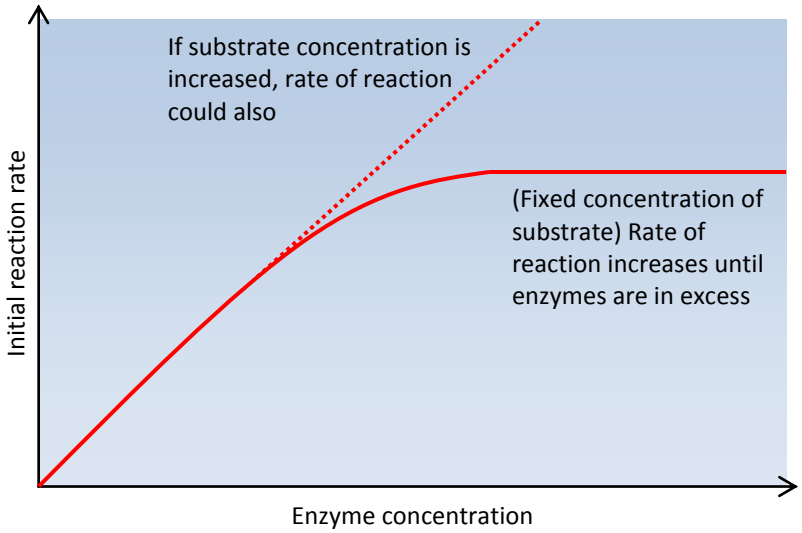
There are two things to consider here: we can change the concentration of the *substrate*, or change the concentration of the *enzymes*. Either way, the reaction rate will change…



▲ Graph to show the relationship between substrate concentration and reaction rate; note that the concentration of enzymes is fixed

First of all, let's consider changing the concentration of the substrate for a *fixed concentration of enzyme* molecules. It goes without saying, if there is no substrate present, the enzyme-substrate complex cannot form – no reaction. So quite obviously, the more substrate there is, the more collisions there will be and so there will be more reactions between the substrate and the active site. But there will be a point at where the rate of reaction reaches its top value (V_{max}). The rate cannot increase any more than this point, because *all* the enzymes present are working at their fastest possible rates, forming enzyme-substrate complexes and releasing product. We say that at this point, the substrate is in **excess**.

We can also examine what happens when the concentration of enzymes changes over a fixed concentration of substrate molecules. A similar relationship would be observed. As the enzyme concentration increases, more active sites become available to form enzyme-substrate complexes with substrates. As more and more of them form, the reaction rate increases. But again, adding more and more enzymes, you will eventually reach a point where all the substrate molecules are occupying enzymes' active sites, so the *enzymes are in excess*.



▲ Graph to show the relationship between enzyme concentration and reaction rate

The graph shows (with the *solid line*) this relationship. The line levels off when the enzymes are in excess, i.e. there are free enzymes laying around with no substrate to use.

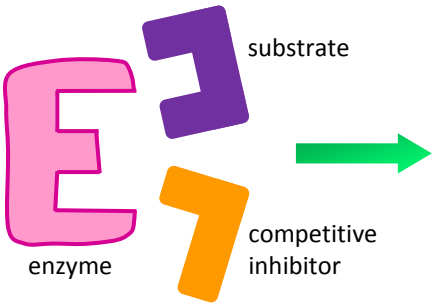
But the V_{max} of this can be increased by altering the substrate concentration. If you increase the number of available substrate molecules, the free enzymes will be able to react with them, therefore the rate of reaction will continue exponentially so long as the appropriate substrate concentration increases alongside it.

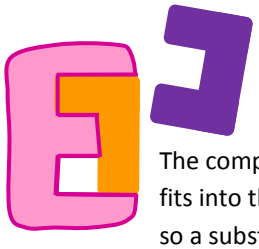
Because the concentration of substrates stops the reaction rate increasing in the graph, we call it the **limiting factor**.

Enzymes and inhibitors

There is one final factor to consider. An **inhibitor** is a substance which reduces the rate of an **enzyme-controlled reaction** by affecting the enzymes involved in some way. Some inhibitors change the shape of the active site, whereas others affect other parts of the molecules, which eventually will affect the active site, *inhibiting* its function.

A **competitive inhibitor** is a molecule which has a similar shape to the substrate required by an enzyme. This enables it to fill the active site, forming the **enzyme-inhibitor complex**. However, because the inhibitor is not identical to the substrate the enzyme is used to, no product is formed, and no reaction is induced. Inhibitors reduce reaction rate because when an inhibitor occupies the active site, a substrate cannot – so fewer reactions can happen.



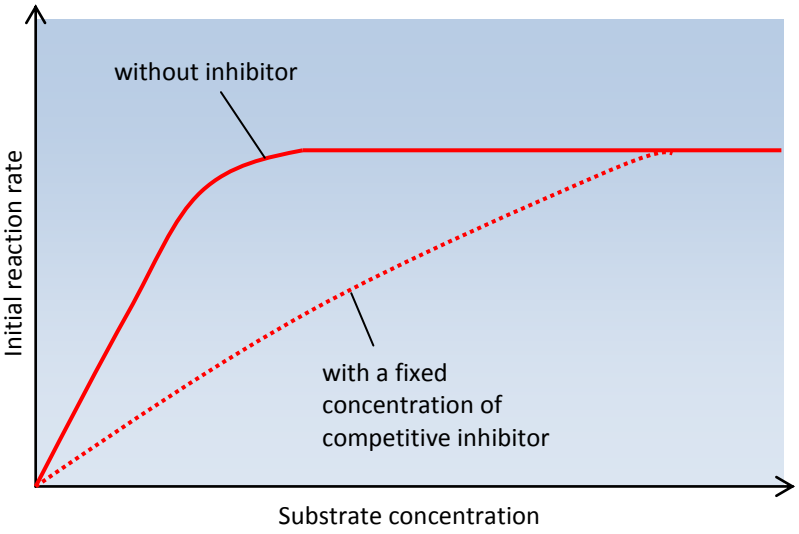


The competitive inhibitor fits into the active site and so a substrate cannot enter

The rate of inhibition depends on the relative concentration of substrate and inhibitor molecules – more inhibitor molecules present mean more collide with the active sites and inhibit substrates from entering them

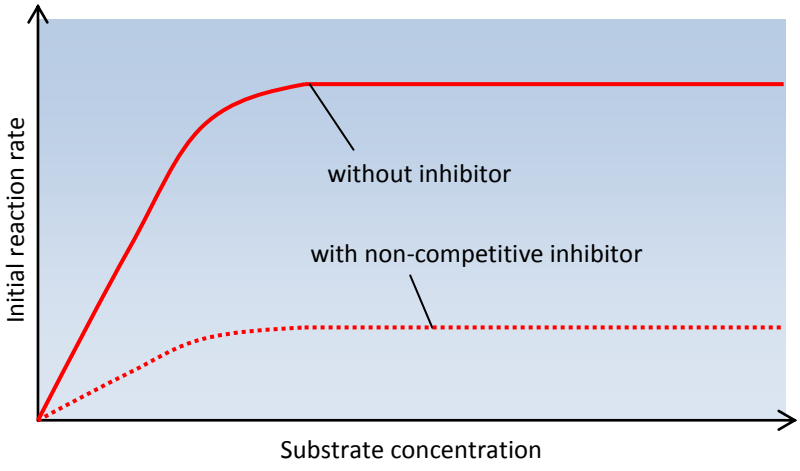
The diagrams show how competitive inhibitors work, and explain how the level of inhibition is caused by the concentration of inhibitor molecules, compared to the concentration of substrate molecules.

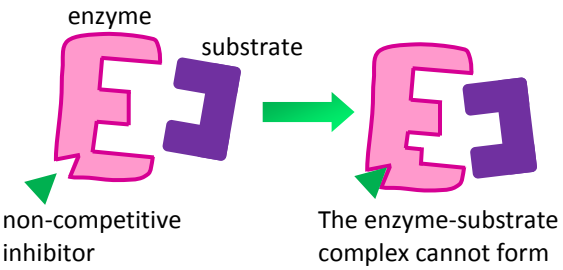
The graph displays the relationship of the competitive inhibitor on the rate of reaction. Increasing the concentration of the substrate would essentially *dilute* the effect of the inhibitor, because it increases the number of collisions between substrate and active site, and decreases the number of collisions between inhibitor and active site.



There is another type of inhibitor, called a **non-competitive inhibitor**. These, as the name suggests, do not compete with substrate molecules for a place in the active site. Instead, they attach to another region.

Non-competitive inhibitors attach themselves to a part of the enzyme away from the active site. This distorts the tertiary structure of the enzyme, and in turn the active site, preventing substrate molecules form being able to enter it. This of course, reduces the rate of reaction.





Most competitive inhibitors do not bind permanently to the active site of an enzyme. They will tend to remain there for a short period and then leave. However, many of the non-competitive inhibitors will bind to an enzyme permanently (**permanent inhibitors**).

If an enzyme is permanently-inhibited by an inhibitor, it is effectively *denatured*.