The effect of enzyme concentrations on the reaction time

My aim of this investigation is to investigate the effect of enzyme Urease concentrations on reaction time. Urease is the enzyme used to breakdown urea into carbon dioxide and ammonia.

I have chosen to carry out this investigation because there are many different areas of biology linked with this investigation that are of interest to me. In addition, enzymes are one of the most important and fascinating molecules found in the body and I would enjoy learning more about the fascinating compound for my own pleasure.

Introduction:

Enzymes are biological organic catalysts. They are specialised globular proteins with a complete tertiary structure that give it the property of being specific for one biochemical reaction that takes place inside every living cell. Many reactions that take place inside living organisms are very slow without the presence of an enzyme. Enzymes act as biological catalysts that allow reactions to take place rapidly in conditions that are found inside living cells without being chemically used up or changed themselves. Each different type of enzyme has a unique three-dimensional structure that enables the enzyme to catalyse one type of reaction. They may affect one particular biochemical reaction strongly but leave a similar reaction unaffected.

In an enzyme catalysed reaction, the reactance, also known as the substrate, binds to the enzyme at specific points known as the active site to form an enzyme-substrate complex. The precise shape of the acitive site is important inorder for the substrate to combine to the enzyme as the substrate is the complementary shape to the active site. The diagram below represents this:

The overall three - dimensional shape of a polypeptide chain is referred to as the enzymes tertiary structure. In globular proteins, the polypeptide chains are tightly folded to form a spherical shape. Many globular proteins are folded so that their hydrophobic groups are on the inside of the molecule and the hydrophilic groups face outwards, making these proteins suitable in water. Globular proteins include enzymes, antibodies and many hormones.

The precise three - dimensional shape of a globular protein molecule determines its function. Therefore, every coil twist, bump and indentation is very important and unique to the enzyme. Various intermolecular bonds including ionic bonds, hydrogen bonds disulphide bonds, and hydrophobic interactions (interactions between non-polar, water repellent groups in the protein) maintain the shape of the enzyme. The tertiary structure of a protein structure together can only form if the correct amino acids are at specific points along a polypeptide chain.

Hydrogen bonding holds molecules together more strongly than other types of intermolecular forces but is weaker than covalent bonding. For hydrogen bonding to occur a hydrogen atom needs to be attached to a highly electronegative atom that needs to be small, and with at least one lone pair of electrons for the Hydrogen atom to interact with. The small hydrogen atom has a greater affinity for the lone pair of electrons on the hydrogen atom so can get very close to the atom making the attraction between the molecules greater.

Once the substrate has reacted in the active site, the enzyme substrate complex becomes and enzyme- product complex and a product if formed.

The prodct then leaves the active site on the enzyme and the process begins again. The equation and diagram below show this:

In order to form products, bonds must be broken in order to form new ones. This requires energy, as bond breaking is an endothermic reaction. The reacting molecules must have enough energy between them to overcome the activation enthalpy barrier before a reaction can take place. The activation energy is the minimum energy that two molecules need in order to react when they collide. It is the energy that must be supplied to enable bonds in the reactants to stretch and break as new bonds.

If the activation enthalpy is high then only a few pairs of molecules will have enough energy to overcome the energy barrier and react to form products, so the reaction will be slow. Catalyst work by lowering the activation enthalpy of a reaction and so they also increase the value of rate constant, k. They do this by providing an alternative reaction pathway for the breaking and remaking of bonds that has a lower activation enthalpy. So in an enzyme-catalysed reaction, reactants need less energy before they can turn into products than they do in an uncatalysed reaction. Their activation enthalpy is lower therefore; the reaction takes place more quickly. The diagram below represents how this works.

Enzymes as proteins are sensitive to changes in their environment. Temperature and pH changes can cause changes in the shape of the enzyme molecule and will therefore affect its activity. Changes in the concentration of both the enzyme and its substrate will also affect the rate of an enzyme-catalysed reaction.

Temperature has a complex effect on the rate of reaction. An increase in temperature increases the kinetic energy of an enzyme and substrate molecules causing them to move more rapidly. This will in turn cause more collisions to take place between the substrate and enzyme molecules, resulting in more enzyme-substrate complexes to be formed. Heat provides activation energy for the enzymes and therefore chemical reactions are more likely to occur at high temperature therefore increasing the rate of reaction.

However, a further increase in temperature can affect the stability of the enzyme molecule. High temperature can cause vibrations to become more violent and so the intermolecular forces that hold the structure of the molecule together are broken and enzyme activity is lost. The precise 3d structure of the active site is disrupted, preventing the enzyme-substrate complexes form forming resulting in a decrease in rate of reaction. The diagram below shows a typical graph for the reaction time against temperature:

The optimum temperature of the enzyme is the teperature in between the two factors at which the enzyme functions properly. At temperature above the optimum temperature the enzyme becomes denatured. This is when the bonds holding the shape together are broken. The primary structure is retained, however the polypeptide chains unravel and loose their specific shape. Once an enzyme has denatured it is unable to bind with a substarte and has therefore lost its catalytic properties. Denaturation is nearly always irreversiable it can also be cused y changes in pH and salt concentration.

Most enzymes have and optimum pH at which they function most effectively. At extremes of pH values, the enzyme molecule may become denatured. This is due to the changes in pH that affect the ionisation of the side groups in the amino acid residues. This effects the overall shape of the molecule and the efficiency of the enzyme-substrate complex formation decreases the rate of reaction. The diagram below shows a typical graph for the reaction time aginst pH:

Changes in both substrate and enzyme concentration also effect the rate of reaction. An increase in enzyme concentration, increases the number of active sites, therefore more enzymes-substrate complexes can form. This increases the rate of reaction, provided there is an excess of substrate molecules.

The rate of reaction depends on the rate of formation of enzyme-substrate complexes. So if the substrate concentration increases, the rate of reaction will increase until there are no more enzymes to bind to. This will result in the rate reaching a maximum velocity and remains constant as the enzymes will prevent an increase in rate. The diagrams below show the effects of enzyme concentration on reaction time and the effect of substrate concentration on reaction time:

For my investigation, I have chosen to investigate the activity of the enzyme Urease. The enzyme Urease is present in many simple organisms. It occurs in many bacteria, several species of yeast and a number of higher plants. Two of the best sources of Urease are jack beans (canavila eniformis), and bacillus pasteui.

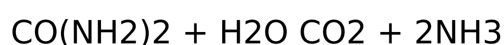
Soya beans (and other legume beans) are normally toasted to make them fit for human consumption. This is because Soya contains substances called Trypsin inhibitors that can cause gut upset to people and animals if they were to eat raw Soya beans. These are difficult to test in the laboratory but Soya also contains the enzyme Urease (relatively harmless compared to Trypsin inhibitors). Various studies have shown that Urease is inactivated at the same rate as Trypsin inhibitors by the toasting process. So if the Soya tests low for Urease activity it means they have been toasted properly to remove Trypsin inhibitors.

Urease enzyme occurs throughout the animal and plant kingdom but jack beans seem to be one of the richest natural sources of Urease enzyme. Urease converts urea + water to carbon dioxide + ammonia (NH_3). This is quite an important reaction in nitrogen metabolism. Urease is high in plants that are legumes (nitrogen fixers) and whose seeds are rich in protein.

Urease activity in soil (from micro organisms in the soil) is also an important part of the nitrogen cycle in ecology. Urease activity is also an important activity of some important pathogenic bacteria. This is because the ammonia produced by the infecting bacteria is toxic and an irritant.

The enzyme Urease catalyses the hydrolysis of toxic urea into ammonia and carbon dioxide. This can be shown in the balances equation below:

Urea + water carbon dioxide + ammonia



$CO(NH_2)_2 + H_2O$ $CO_2 + 2NH_3$

Urea is one of the compounds that make up nitrogenous waste in mammals. Breakdown of access amino acids during deamination produces ammonia. Ammonia is a very toxic and can only be safely excreted if large amounts of water are available to dilute it to safer levels. This is not possible in aquatic or terrestrial or animals such as mammals. These mammals convert the ammonia in the body to urea, which is much less toxic. Urea is a very small molecule and is soluble in water. It diffuses readily across cell membranes and may be found in small amounts in most body fluids.

The production of ammonia raises the pH of the solution and can be detected by using a suitable acid base indicator.

Urease is active over a wide range of pH so any number of indicators can be used provided that the original solution is buffered at the right level to start with.

There are two different types of indicators that I am going to use for this experiment, phenolphthalein and Bromothymol Blue. Phenolphthalein changes from colourless to pink at quite a high pH9. At these pH's a small amount of ammonia doesn't affect the pH very much so the transition from clear to pink occurs only very slowly. As a consequence, large variations can occur in determining the first faint pink coloration required for the end-point.

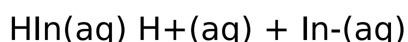
For this reason I am also going to use Bromothymol Blue. This is a better indicator of the two as the colour change, yellow to blue, occurs around pH6 to pH8. The midpoint colour, turquoise, occurs at about pH7. 5. At these pH's a small amount of ammonia will produce a large change in pH so the end

point occurs very quickly. This leads to little variation in the timing of the end-point.

A buffer solution is a solution that contains a weak acid and a salt of a weak acid. Depending on the relative concentrations of the weak acid and the salt, the solution will have a characteristic pH value. The term pH refers to the relative concentrations of H+ ions in a solution. The pH is the negative of the logarithm of the molar concentration of H+ ions. A solution is classed as an acid if there is a high concentration of H+, which indicates a low pH value, (below pH7). Similarly a solution is classed as a base if there is a low concentration of H+, which indicate a high pH value, (above pH7). PH7 is neural, neither acid nor base.

Buffer solutions are have an important role in maintaining a particular pH value of a solution, as they resist changes in pH. They do this by neutralising a solution. If more acid is added to a buffer solution, which tends to decrease the pH as by adding an acid to the buffer solution increases the concentration of H+ ions, the excess hydrogen ions are effectively neutralised by the salt of the weak acid. If a base is added to the buffer solution, which will increase the pH as by adding a base to the buffer solution decreases the concentration of H+ ions, the lack of hydrogen ions are neutralised by the weak acid by supplying extra ions.

Acid - base indicator are organic substances that are weak acids themselves. When a reaction is complete, an indicator changes colour to determine the end-point of the titration. Generally, this can be represented by the following equation:



HIn(aq) H+(aq) + In-(aq)

Acid Base

A change in pH causes a shift in equilibrium, which causes a colour change of the acid, or its conjugate base. A dramatic colour change shows the presence of a good indicator, as it is clearer to detect the end-point of a reaction.

Acids and alkalis can be classified as strong or weak depending on the extent to which they form ions when dissolved in water. Strong acids and alkalis are completely in the form of ions in a dilute solution, whereas for weak acids and alkalis, this process is only partially complete. I will make sure that I only use enough of the indicator to give an observable colour change.

Hypothesis

As the enzyme concentration of Urease increases the reaction time of urea broken down into ammonia and carbon dioxide will increase. To prove my hypothesis I will be using the spearman rank correlation test and will be looking for a positive correlation. I will be taking 12 results of each experiment to generate useful, accurate, reliable concordant results and to provide suitable data for analysis of the spearman rank correlation test.

Requirements

Below is a list of all the apparatus and chemicals I will need for my experiments and the reasons for my choices. Before using my apparatus I

will make sure that everything is thoroughly cleaned first and rinsed with distilled water to remove any impurities, and dried out thoroughly.