

# The effects of temperature on catalase in yeast and liver



As stated in the headline, this will be a plan to investigate the effects of temperature on the enzyme catalase in yeast and liver. But before I go in to detail about the experiment itself, the biology behind the reaction should be discussed. Starting with what will in great part control the reaction, some knowlegde of the biology of enzymes should be obtained. As we have learned, enzymes may be described as globular proteins with catalytic properties. They cannot cause reactions to occur, but only speed up ones which would otherwise take place more slowly. While the enzyme molecule is normally larger than the substrate molecule it acts upon, only a small part of the enzyme, called the active site, actually comes into contact with the substrate.

It is commonly accepted that the enzymes operate on a socalled lock and key mechanism. The lock and key mechanism works in the way that in same way as a lock fits a key, the substrate fits exactly into the active site of the enzyme molecule. There, the substrate is either split into two or more molecules, or two or more molecules may be joined together, as with dipeptide. During the entire process, the enzyme remains unchanged and maintains its initial shape. This allows it to keep turning sustrate into products, being used over and over again.

The rate at which enzymes normally work can be shown on a graph as a rapid increase at first, as there is plenty of substrate for the enzymes to bump into and turn into product. At a point though, the reaction will reach its peak. A maximum number of substrate is being turned into product per second/minute. After this, the rate of which the process is taking place will decrease. With fewer substrates around for the enzymes to work on, the <https://assignbuster.com/the-effects-of-temperature-on-catalase-in-yeast-and-liver/>

collisions are fewer and longer apart. After a while the process will come to a full stop as there are no more substrates to be turned into a certain product.

What you should doAlso involved in forming products is the activation energy. This is the energy needed in many reactions to convert a substrate to a product. When a substrate locks to the active site of an enzyme, the shape of its molecule changes, which makes it easier to change into a product. The activation energy with enzyme is lower, making it economical to use as energy can be saved and the enzymes can be re-used. Different factors can influence the working rate of an enzyme.

These include enzyme concentration, substrate concentration, pH and temperature. As said above, we will be investigating the effects of the latter in this experiment, but we still have to take into account the possible influences of the others.> The effect of enzyme concentration: The more enzyme present, the more active sites will be available for the substrate to lock into. The initial rate of a reaction will increase proportionally with enzyme concentration as long as there is plenty of substrate available. When there is no more substrate available to fit the lock and key, the addition of more enzymes will make no difference. There is simply no substrate available for them to collide into.

> The effect of substrate concentration: The more substrate molecules there are, the more often one can lock into the active site of an enzyme. In other words, as substrate concentration increases, the initial rate of reaction also increases. This is only up to a certain point though. As we go on increasing the substrate concentration, keeping the amount of enzymes the same,

there comes a point where every enzyme's active site is working continuously. It cannot take on any more substrate at the present time, and the substrates literally have to "queue up". At this point, we say the enzyme is working at its maximum possible rate, known as  $V_{max}$ .

; The effect of pH: Most enzymes work best in neutral conditions, which is a pH of around 7. Some, for instance pepsin, have a different optimum pH, but they are the exception rather than the rule. pH is a measure of the concentration of H-ions in a solution, The higher the pH, the lower the H-ion concentration. A pH which differs greatly from the optimum pH could cause denaturation of an enzyme. The active site only needs a slight change to become unfittable with the substrate, which could happen as the H-ions interacting with the R-groups in the protein affect the way in which the amino acids bond with each other- in other words change their 3D shape.

; The effect of temperature: As temperature increases, the enzyme and substrate molecules move faster. At normal rate, few collisions will occur due to the low speed at which the molecules move about. However, as the temperature rises, collisions happen more frequently so the substrate will lock on to the active site of the enzyme more often. Also, the collisions are richer in energy, making it easier for bonds which need to be broken in order for the reaction to occur to break apart. Up to a certain point, the rate of reaction will increase as temperature continues to do so.

This point is referred to as optimum temperature. Optimum temperature for enzymes varies, but most human enzymes have an opt. temp. of around 40 celcius. Above that point though, the structure of the enzyme molecule

vibrates with such energy that some of the intermolecular bonds holding the enzyme in its precise shape begin to break. The shape of the active site changes along with the loss of the molecule's activity.

The enzyme is said to be denatured. Diagram: In the above diagram, 3 different temperature optimums are illustrated to show the varying optimum temperatures of enzymes. As you can see, the curve varies slightly as well. The blue line might represent the enzyme of a marine animal in an arctic environment, the red could represent an enzyme in the human body and the green an enzyme found in organisms in for instance hot springs or volcanic areas. Another way of using the enzymes efficiently is to immobilise them, in other words sticking them to a specific surface over which substrate can be passed, using the enzymes over and over again also avoiding enzyme-containing products.

In this experiment though, we will not be using immobilised enzymes, but the enzyme catalase which is found in yeast and liver.\* A bit about the enzyme catalase: Catalase is an intracellular enzyme, meaning that it works inside the cell. It obtains the highest known "turn-over" rate, by which we mean the number of substrate molecules which an enzyme can act upon in a given time. In fact, catalase can bind with hydrogen peroxide molecules, split them into water and oxygen and release these products at a rate of  $10^7$  molecules per second. As catalase is found in liver, a mammal organ, we would expect the optimum temperature to be between 35-40. The optimum temperature might be slightly different in yeast though, as this is not naturally found in mammals.

This is another thing we will establish from this investigation. As said above, catalase splits hydrogen peroxide into water and oxygen. Hydrogen peroxide itself is, apart from being a byproduct of metabolism, toxic. It is also a powerful oxidising agent.

The balanced reaction, catalase acting as a catalyst, is shown below:  $\text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$  For every 2 moles of hydrogen peroxide broken down in the reaction, 1 mole of oxygen will be created. Seeing as 1 mole of gas occupies 24 dm<sup>3</sup> under standard conditions (room temperature and pressure) which would prove hard to measure with our instruments, the H<sub>2</sub>O<sub>2</sub> used in the reaction should be limited to small amounts. Variables: \* Constant variables are the ones which will remain the same during the entire experiment. In this case mainly represented by the yeast and the liver, and the substrate. The yeast and liver will be homogenised and used as a yeast/water and liver/water suspension. One reason for doing this is to secure an even enzyme concentration.

If we were to use single cut pieces, a piece cut from the center of the yeast/liver may have higher enzyme concentration than one in the outer edges. The best way of keeping this a fair test is in other words to make a suspension, using for instance 1g liver and 50cm<sup>3</sup> water. Mixing the sample of yeast/liver will make possibly different enzyme concentrations blend in together. Also, dried yeast ages more quickly, becoming denatured and deteriorated. Another important factor is to use the same yeast or liver throughout the entire experiment. If we were for instance to suddenly use a new, fresh piece of yeast, this could contain a higher concentration of enzymes than the original.

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Seeing as a higher concentration of enzymes would produce more substrate at a quicker rate, this would make the experiment inaccurate. Another necessity in order to make this a fair test is in other words to use the same sample of yeast/liver throughout the experiment. As for the substrate, we need to use the same concentration and volume for all samples. The way in which we can make sure of this is to make out one mixture of H<sub>2</sub>O<sub>2</sub>, for instance 50 cm<sup>3</sup>, and use fractions of this for all the samples.

The opposite, making new concentrations for each sample would make this variable anything but constant. Changing the concentration would affect the results, as a higher or lower concentration would produce different amount of products at different speeds. Important for both is to keep a constant volume. Difference in substrate or enzyme volumes would as explained above have serious impacts on the experiment.

To maintain the same volumes we will measure both suspension and substrate as accurately as possible in 10cm<sup>3</sup> measuring cylinders. Another variable in enzyme reactions is pH. There is no reason why the pH should vary in this experiment though, as we will use the same substrate and the same suspension in all samples, only changing the temperature. Therefore, I will not expect pH as a variable to be a factor that needs to be taken heavily into account in this investigation.\* Independent variable is the one we will vary.

In this investigation the independent variable is temperature. In order to get a wide range of measurements, we will use waterbaths with temperatures starting from 10 degrees C, increasing by 10 degrees C (20, 30 etc) up to 60

degrees C. We will in other words investigate the process at 6 different temperatures. This procedure is necessary to obtain the wanted range of results which is needed to plot a realistic graph.

A graph showing only a couple of points would not give enough information. Also, it is needed to observe the full reaction taking place, not only what is happening every half an hour or so. With enzymes working at the rate of catalase, this would not be sufficient enough. I have chosen waterbaths because they will incubate the solution, keeping it at a constant temperature. Left in open air it would soon cool off/ heat up, changing the temperature and thus the working rate of the enzymes. Also, for this reason the solutions must NOT be removed from their waterbaths.

\* Dependent variable is affected by/dependent on the independent variable. In this case this will be oxygen. As the temperature rises and the rate of reaction increases, we would expect the amount of oxygen being released to increase as well. Likewise, as the temperature exceeds the optimum temperature for the enzyme and the rate of reaction decreases we would expect the amount of oxygen being released to decrease as well. There are different ways of measuring the amount of oxygen gas being emitted.

One, the simplest, is to simply count the bubbles. Scientificly though, this alone is not particularly accurate. Another is to use a gas syringe, and a third to use an inverted burette filled with water. A combination of two of these is also possible, illustrated as below: This is the method we will be using in our investigation. As we can see, this allows both the released bubbles to be counted and the volume to be measured in the burette.

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