Effect of enzyme concentrations on oxygen production

The function of an enzyme is explained by the lock and key theory: the active site of an enzyme (the lock) has a specific shape in which only the precise amount of substrate (the key) will fit – forming an enzyme-substrate complex (the product).

Catalase can speed up the decomposition of hydrogen peroxide as the shape of its active site matches the shape of the hydrogen peroxide molecule. This type of reaction is an anabolic reaction (when a molecule is broken down into smaller molecules).

Enzymes are able to increase the rate of reaction without actually being consumed in the process. Small quantities at low temperatures are able to produce results, which would normally require high temperatures and a violent reaction from any normal chemical means. Although increases in temperature may speed up the reaction, the heat will also denature the enzymes and make them unstable. All enzymes are catalysts (a substance that causes or accelerates a chemical reaction without itself being affected), and they work best at pH7.

As long as the concentration of the enzyme substrate (hydrogen peroxide) is much higher than the enzyme (catalase) concentration, the rate of reaction is directly proportional to the concentration of the catalase. This is because, as the enzyme concentration rises, the number of active sites that are available to interact with the substrate also rises; this increases the rate of product formation.

My original experiment was an investigation into how the temperature of yeast would affect their rate of respiration. However when it came to the

actual experimentation we found that the volume of dye and the volume of yeast we were using was too great (resulting in the dye actually rising out of the ‘ U tube’): this meant that I would have to scale these down. However, we soon found that by decreasing these volumes the results produced were very small so I decided to completely change my experiment; instead of testing temperature, I decided to change the concentrations of the yeast I was using, and see how that would have an effect on the yeast’s rate of respiration and therefore the volume of oxygen evolved.

Key Variables

Concentration of yeast: The rate of respiration in yeast (and therefore the volume of oxygen evolved) may change depending on its concentration

Volume of hydrogen peroxide: I am mixing this with the yeast so the catalase will cause it to decompose into water and oxygen

Type of yeast: The rate of respiration may vary in different types of yeast

Temperature of the room: the temperature can affect the rate of respiration for the yeast depending how hot or cold it is

Type of equipment: the length of the glass delivery tube can affect the volume of oxygen evolved

Volume of yeast solution: The volume of oxygen evolved in yeast may differ depending on the volume of yeast solution

Independent Variable

Concentration of yeast: I am investigating how the volume of oxygen evolved from yeast (specifically the enzyme in the yeast – catalase) changes when the concentration of yeast suspension varies so it is important to change this variable

Controlled Variables

Volume of hydrogen peroxide: since I am already changing the concentrations of the yeast I use, I must keep the volume of hydrogen peroxide the same throughout in order to make it a fair test

Type of yeast: I must use the same type of yeast throughout: otherwise this could affect the amount of oxygen evolved in the yeast

Temperature of the room: I am going to maintain the same temperature in the room I am conducting my experiment in to try and get the most accurate result I can

Type of equipment: the size and diameter of the glass delivery tube affects how the long the test lasts – ultimately having an effect on the volume of oxygen evolved

Volume of yeast solution: I have to keep this the same if I want to get accurate results

Dependent Variable

Volume of oxygen evolved: The volume of oxygen evolved will change depending on how much concentrated yeast is being mixed with the hydrogen peroxide

My prediction:

I think that as I increase the concentration of yeast, the amount of oxygen evolved will increase proportionally: this is because as the enzyme concentration rises, the number of active sites that will be available to interact with the substrate (hydrogen peroxide) also rises – increasing the rate of oxygen evolved.

My experiment

Apparatus used:

- Gloss delivery tubes
- Screw clip
- Clamp
- Rubber tubing
- Litre beaker
- Inverted barrel
- Boiling tube
- Plastic syringes
- Rubber bung
- Glass stirring rod
- Stopwatch
- Experimental Procedure

First, I used clamps to support the boiling tube and attached the rubber tubing to the barrel of the 20cm3 syringe

Then I removed the plastic syringe, leaving the needle in the same position, and removed the bung from the boiling tube

After stirring the yeast suspension (which I made by adding 10g dried yeast to 100cm3 water I prepared it one hour before I actually needed to use it), I used a plastic syringe to introduce 5cm3 of yeast to the boiling tube

I then filled the 1cm3 syringe with and placed it into position

I opened the screw clip to draw water into the barrel of the 20cm3 syringe and closed it once the barrel was full, then I injected the hydrogen peroxide into the boiling tube

I measured and recorded the volume of oxygen collected in the barrel of the 20cm3 syringe over a period of five minutes (I also used a stopwatch to measure how much oxygen was evolved per minute)

This was repeated using 10, 15, 20, 25 and 30cm3 yeast suspension in the boiling tube (with fresh samples of yeast and hydrogen peroxide)

This method was repeated for the above three times and a mean average was calculated; my results were recorded in a table (see my results)

Using the tabulated data I plotted graphs of my results before analysing them

In the above table we can see that when 5cm3 of yeast is being mixed with the enzyme substrate and an average of 4. 77cm3 of oxygen is being evolved, then in theory when 10cm3 is being mixed with the enzyme substrate the volume of oxygen evolved should be double the average volume produced for 5cm3 of yeast (4. 77cm3 x 2 = 9. 54cm3). However, this is not the case, as actually an average of 5. 33cm3 of oxygen is being

evolved for 10cm3 of yeast being mixed with the hydrogen peroxide: this is because part of the oxygen evolved is actually being used by the substrate for respiration – this results in the curve of the line in graph 3 + 4.

Data analysis of all graphs

In graph 1 a pattern can be seen in the results: the higher the yeast concentration, the greater the volume of oxygen is evolved. We can also see that towards the end of run 1 the volume of oxygen produced does not change and it becomes a straight line: this might be attributed to the fact that the yeast has become saturated with the substrate. In my scatter graph I have decided to use polynomial lines of best fit – this is because rather than a linear line of best fit (which is completely straight and does not actually show the curve/steepness of the varying results) a polynomial line actually shows the curve, and allows the viewer of the graph to see how the production of oxygen actually fluctuates and changes.

In graph 2 we can see that most of the oxygen evolved from the reaction passes into the collecting vessel within one minute of mixing the two reactants together. Afterwards the rate slows and only a small volume of oxygen is produced afterwards (particularly in between the third and fifth minutes). The pattern of oxygen evolution indicates that the reaction is extremely rapid.

In graph 3 it can be seen that as I increase the concentration of yeast the volume of oxygen evolved increases proportionally: this is because as the enzyme concentration increases, the number of active sites that are

available to interact with the hydrogen peroxide molecules also rises – thus raising the production of oxygen.

In graph 4 we can see that the error bars are very small, which means that the results produced must be very accurate (as there is not much range between the different volumes of oxygen produced per yeast suspension).

Conclusion

Overall, my results show that there definitely is a quantitative relationship between the concentration of yeast/catalase, and the volume of oxygen evolved: the higher the yeast concentration, the higher the volume of oxygen was evolved: this was because as the enzyme concentration rose, the number of active sites that were be available to interact with the substrate (hydrogen peroxide) also rises – increasing the rate of oxygen evolved; hence, my original prediction was correct.

Evaluation

All in all I would say that my experiment was a success as I had no anomalous results (so I would not need to repeat any), and my results agreed with my prediction; my results were also substantial enough to let me draw a conclusion from them. I would say that my experiment was kept fair, however I believe that more could have been done to make sure my results were of optimum accuracy: for one I could have regulated the temperature of the laboratory I conducted my results in (maybe by having a thermometer with me and making sure that the temperature more or less stayed the same). Also, when measuring the volume of oxygen evolved per minute, the results maybe could have been more accurate (as sometimes there was a

delay in pausing the stopwatch, causing more seconds to be added onto the actual time taken). However, as seen in graph 4, the error bars are very small, meaning that the accuracy of my results were very precise: this is most probably due to the fact that I repeated the experiment for each of my yeast concentrations three times so I could have lots of results to back up my prediction/conclusion.

If I had to make any modifications to my experiment, one would be that I covered a larger range (in terms of yeast concentration) so that I could have even more results to back up my conclusion; however I don't think this would be a necessary change as I believe the results I have already firmly support my conclusion.

Sometimes when I was measuring the volume of oxygen evolved per minute (for a period of five minutes) I sometimes experienced difficulty in stopping the stopwatch as soon as one minute had passed: maybe if I had had two people timing separately for me, I could have ensured that the final recorded time was accurate. Apart from that though, the rest of my equipment succeeded in making my experiment a ' fair test' – the syringes had a set amount of substrate in them, thus resulting in me correctly injecting the precise volume of hydrogen peroxide each time.

I would not make any improvements to my method other than washing each syringe after use, to prevent any chance of contamination.