Steven Burnett IB Yr1 IB Biology Coursework 03/01/2011

Investigation to find the effect of substrate concentration on the rate of an enzyme controlled reaction

Design

Aspect 1: Defining the problem and selecting variables

Investigation title: is the rate of an enzyme controlled reaction affected by substrate concentration?

Background information

Enzymes are often referred to as "biological catalysts", since they increase the rate of reactions in living organisms. Enzymes are also substrate specific, which means that only a specific substrate can fit into its active site (which is where the catalysing effect occurs) and as such, there are different enzymes which deal with different reactions (for example, protease breaks down proteins into amino acids) – this is the basis for the 'lock and key' theory. The stages of an enzyme controlled reaction are:

1) Enzyme + substrate

The enzyme and substrate are in solution together

2) Enzyme substrate complex

A substrate has moved into the active site

3) Enzyme product complex

The reaction has taken place, but the products haven't been released from the active site

4) Enzyme + product

The enzyme and product are now in solution together (after the product was released from the active site)

The rate of an enzyme controlled reaction are mainly affected by: temperature, substrate concentration, enzyme concentration and pH. By increasing the substrate concentration, there is a higher chance of a substrate colliding with an enzyme and subsequently reacting (as there is simply more substrate moving around in the solution). Increasing the substrate concentration further will cause the rate of reaction to plateau when all of the enzymes' active sites are constantly in use – at which point the enzyme concentration can be considered a limiting factor.

Catalase, the enzyme that will be used, is an enzyme found in nearly all living organisms and it catalyses the breakdown of hydrogen peroxide into water and oxygen. The balanced equation:

$$2H_2O_2 > 2H_2O + O_2$$

The investigation will involve soaking a circular piece of paper in catalase, dropping it into a hydrogen peroxide solution and then timing how long it takes the piece of paper to rise to the surface (oxygen bubbles will be produced, which are less dense than hydrogen peroxide. So, when enough oxygen is produced, the piece of paper will be carried to the surface).

Hypothesis

Changing the substrate concentration will have an effect on the rate of an enzyme controlled reaction (as detailed previously).

Null hypothesis

Changes to the substrate concentration won't affect the rate of an enzyme controlled reaction.

Risk assessment

Glass test tubes/beakers should be handled with care to prevent them from breaking. Any broken glass should be swept up with a dustpan and brush and disposed of appropriately.

Hydrogen peroxide solution must be dealt with safely; lab coats and safety glasses should be worn for the duration for the experiment to avoid contact with eyes and clothes.

Variables
Independent variable: the concentration of the hydrogen peroxide solutions

Dependent variable: the time taken for the piece of paper (soaked in catalase) to reach the surface of the solution after submersion

Control variables (these will be kept constant):
- the size of the pieces of paper
- the volume of the solutions
- the temperature of the solutions
- the pH of the solutions

Aspect 2: Controlling variables
Independent variable: the concentration of the hydrogen peroxide solutions will be changed by adding distilled water to hydrogen peroxide (with the aid of a syringe). The concentrations used will be:
$0.20mol/dm^{-3}$
$0.40mol/dm^{-3}$
$0.60mol/dm^{-3}$
$0.80mol/dm^{-3}$
$1.00mol/dm^{-3}$
Refer to the dilution table (in the method section) for the quantities of hydrogen peroxide and distilled water used to form the solutions.

Dependent variable: It will be recorded with a timer (in seconds) that will be started as soon as the piece of paper enters the solution and stopped as soon as it reaches the surface (after submersion).

Control variables:
- the size of the pieces of paper

The pieces of paper used will be the cuttings from a hole punch – so therefore, each piece will be the same size.

- the volume of the solutions

$10cm^3$ of hydrogen peroxide will be measured out by using a measuring cylinder for each test.

- the temperature of the solutions

The experiment will take place in an air conditioned laboratory, so it can be expected that the temperature will not vary enough over the course of the experiment to have a significant effect.

- the pH of the solutions

1ml of pH7 buffer will be added to the solution.

Aspect 3: Developing a method for the collection of sufficient data
Apparatus
- Hydrogen peroxide $1.00mol/dm^{-3}$ ($150cm^3$)
- Distilled water ($75cm^3$)
- Syringe ($10cm^3$)
- 15 test tubes
- Stopwatch (seconds)
- Pieces of paper
- Hole punch

- 3 petri dishes
- 5cm³ catalase enzyme
- Safety glasses
- pH7 buffer
- Tweezers

Method

Preparing the hydrogen peroxide solutions

Using the table below, prepare the various concentrations of hydrogen peroxide:

Volume of distilled water (cm^3)	Volume of 1mol/dm^{-3} hydrogen peroxide (cm^3)	Concentration of hydrogen peroxide solution (mol/dm^{-3})
8.00	2.00	0.20
6.00	4.00	0.40
4.00	6.00	0.60
2.00	8.00	0.80
0.00	10.00	1.00

Label 5 rows of 3 test tubes with the different concentrations (0.20mol/dm^{-3} to 1.00mol/dm^{-3}) and add 10cm^3 hydrogen peroxide solution into the appropriate test tubes.

Preparing the pieces of paper

Fill three petri dishes with the catalase enzyme solution and soak 5 pieces of paper into each.

Investigating the effect of substrate concentration on reaction time

Using tweezers, take a piece of paper from a petri dish and drop it (from the rim of the test tube) into a 0.20mol/dm^{-3} solution. Start the stopwatch once it touches the solution and then stop it once it floats back to the surface.

Using the same petri dish as before, repeat with the remaining pieces of paper for the 0.40mol/dm^{-3}, 0.60mol/dm^{-3}, 0.80mol/dm^{-3} and 1.00mol/dm^{-3} concentrations.

Repeat the experiment two more times for each concentration (this is done to increase the accuracy of the results and to make anomalous results easier to spot). Dispose of the solutions and pieces of paper once the experiment is complete.

Data collection and processing

Aspects 1, 2 & 3: Recording, processing and presenting raw data

Raw data

Table #1: Time taken for pieces of paper to rise in various concentrations of hydrogen peroxide solution

Concentration of hydrogen peroxide solution (mol/dm^{-3})	Time taken (seconds)		
	#1	#2	#3
0.20	63.0	65.0	58.0
0.40	34.0	34.3	33.8
0.60	18.0	18.3	18.1
0.80	12.2	12.0	12.3
1.00	8.2	8.0	8.2

Processed data

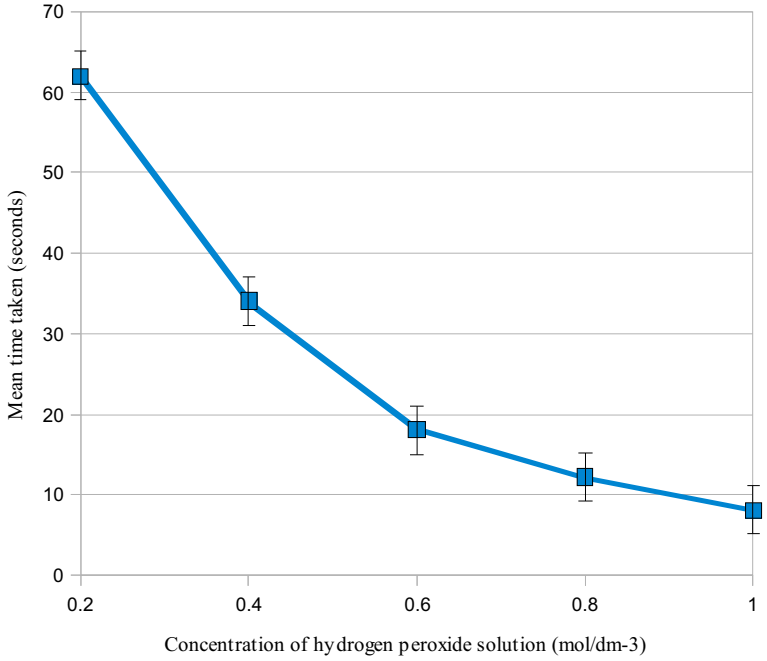
Table #2: Mean time taken for the pieces of paper to rise

Concentration of hydrogen peroxide solution (mol/dm^{-3})	Mean time taken (seconds)	Time range (seconds)	Rate of reaction (arbitrary)
0.20	62	58.0 - 65.0	16.1
0.40	34	34.3 - 33.8	29.4
0.60	18.1	18.0 – 18.3	55.2
0.80	12.2	12.0 – 12.3	81.9
1.00	8.1	8.0 – 8.2	123.5

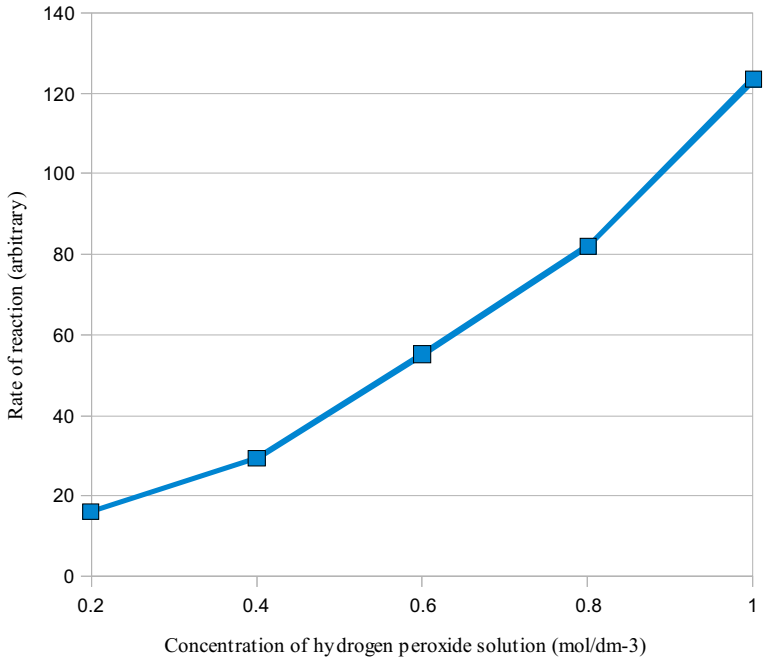
The representative rate of reaction is calculated by the following:

(1/mean time) * 1000

Graph #1: Mean time taken for the pieces of paper to rise in various hydrogen peroxide concentrations



Graph #2: Rate of reaction in varying hydrogen peroxide concentrations



Conclusion and Evaluation

Aspect 1: Concluding

From the results collected in can be concluded that the substrate concentration does affect the rate of an enzyme-controlled reaction as stated in the hypothesis for this investigation.

As the hydrogen peroxide solution increases in concentration, there is an increasing chance for the substrate (the hydrogen peroxide) to collide with the catalase – there is simply more hydrogen peroxide molecules in the same area – which will increase the rate of the reaction as it is increasing likely that more enzymes will be catalysing at any given time.

It is expected that increasing the substrate concentration further will results in the reaction time plateauing when all of the enzymes are in constant use, but my results don't show this effect. If this does occur, then increasing the substrate concentration further still will result in no change in the reaction time; other factors, mainly the enzyme concentration, would therefore be limiting the reaction time.

Aspect 2: Evaluating

Although the experiment supports the original hypothesis, there are limitations:

- Enzyme concentration – each piece of paper used was soaked in the catalase-filled petri dish for a varying length of time. As a result of this, the concentration of the enzyme present on the pieces of paper is uncertain (being soaked for a longer time will allow the piece of paper to absorb more catalase, which would speed up the reaction as there would be more of the enzymes). This does make the investigation less reliable, but the effect would have probably been only slight; however, to improve on this, the length of time that each piece of paper is soaked should be timed and kept the same.
- Timing – although only a minor issues, human reflexes add a small margin of error in the times of each result, since the timing had to start as the pieces of paper touched the substrate and as soon as they touch the surface again. A computerised system could used in place of a human, but this would be costly and impractical to setup.
- The concentrations of hydrogen peroxide didn't show the plateauing effect that was predicted– to improve on this, a greater range of concentrations could be used (for example 0.5M to 2M) which would clearly demonstrate the effect and completely prove the hypothesis.