

Name _____

The Effect of Substrate Concentration on Enzyme Activity

Background

Hydrogen peroxide is a toxic waste product formed as a result of metabolic reactions in cells.

The enzyme catalase catalyses the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. Potato extract can be used as a source of the catalase enzyme.

Introduction

For this practical task you will determine the effect of substrate (hydrogen peroxide) concentration on the activity of catalase in potato extract.

You are given full instructions for the practical procedure, which must be followed carefully.

Before you start any practical work, you are advised to read through the procedure and study Table 1.

It is your responsibility to organise your time efficiently and to work safely. You will be assessed on the accuracy of your results.

Apparatus

You are provided with the following apparatus:

- five tubes
- one 10 cm^3 syringe
- one 1 cm^3 syringe
- glass rod
- watch glass
- forceps
- permanent marker pen or chinagraph pencil, and rule
- timer
- beaker, water and paper towels (to rinse and dry)
- large empty container labelled waste.

Materials

You are provided with the following materials:

- 15 cm^3 potato extract
- hydrogen peroxide at each of five different relative concentrations (2, 4, 6, 8 and 10 arbitrary units)
- small paper discs.

Safety

Hydrogen peroxide is an irritant. Take care when handling solutions that contain hydrogen peroxide. In the event of contact with skin, flush with water.

Part 1

Procedure

Read steps 1 to 13 and study Table 1 before you start any practical work.

- 1 Label the five tubes so that you have one tube labelled for each of the five separate relative concentrations of hydrogen peroxide.
- 2 Use the marker pen and rule to mark a horizontal line on the side of each tube, 5cm up from the bottom of the tube.
- 3 Choose one of the hydrogen peroxide concentrations and add enough of it to the tube labelled with the corresponding concentration so that it reaches the level of the marked line.
- 4 Add 1.0 cm³ of potato extract to the watch glass.
- 5 Soak three paper discs in the potato extract in the watch glass for 30 seconds.
- 6 Use the forceps to remove one of the soaked paper discs from the extract and gently touch the disc to the side of the watch glass to remove excess extract.
- 7 Transfer the disc to the bottom of the tube containing the hydrogen peroxide. If necessary, use a clean glass rod to push the disc to the bottom of the tube.
- 8 Start the timer as soon as the disc touches the bottom. In Table 1, record to the nearest second the time taken for the disc to reach the surface.
- 9 Remove and discard the paper disc.
- 10 Rinse the forceps (and glass rod, if used) in water and dry them on a paper towel.
- 11 Repeat steps 6 to 10 with the two remaining soaked paper discs in turn.
- 12 Rinse and dry the watch glass.
- 13 Repeat steps 3 to 12 with each of the remaining concentrations of hydrogen peroxide.

Table 1

Relative concentration of hydrogen peroxide (arbitrary units)	Time taken for disc to reach surface (s)			
	Repeat 1	Repeat 2	Repeat 3	Mean
2				
4				

6				
8				
10				

Part 2

1 Using the data you recorded in Table 1, calculate the mean time taken for the discs to rise to the surface in each concentration of hydrogen peroxide.

Record the mean times in Table 2 below. [1]

Table 2

relative concentration of hydrogen peroxide (arbitrary units)	mean time taken for disc to rise to surface (s)	relative rate of activity (s ⁻¹)
2		
4		
6		
8		
10		

2 To compare the effect of substrate concentration on the activity of catalase, the relative rate of activity at each concentration can be calculated using the formula:

$$\text{relative rate of activity} = 100 \div \text{mean time}$$

Using the formula, calculate the relative rate of activity at each concentration. Write your answers in Table 2. [1]

3 Use your processed data to draw an appropriate graph of relative rate of activity on the graph paper provided. [3]

4 State the relative concentrations of hydrogen peroxide that gave the slowest and fastest reactions.

Slowest arbitrary units

Fastest arbitrary units [1]

5 Compare the effect of increasing the substrate concentration from 2 - 4 arbitrary units to increasing the substrate concentration from 4 - 8 arbitrary units.

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.....
.....
..... [2]

[Total: 8]

Describing data

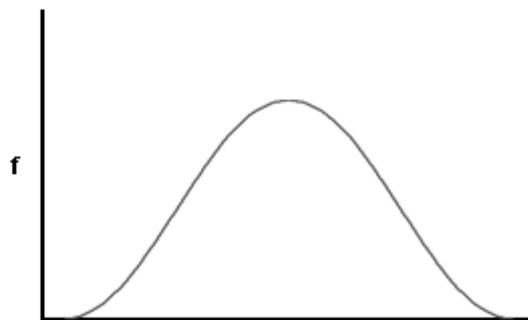
Standard deviation

Using standard deviation is better than simply using the range of data as it **uses all the observations**, and is less affected by the **outliers**.

Low Standard Deviation



High Standard Deviation



A “thin” curve means that most values remain close to the average, and the standard deviation is small.

A “fat” curve means that there is a wider spread of values about the mean, and the standard deviation is large.

How to calculate Standard deviation

The standard deviation is calculated using the formula:

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

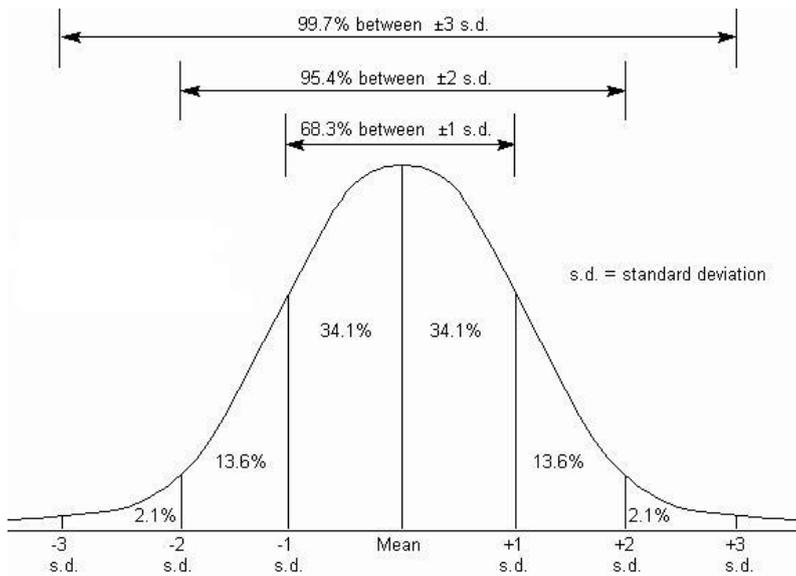
SD = Standard deviation

X = value

\bar{x} = mean

n = the number of values you have

± 2 Standard Deviations



When data has a 'normal distribution' as shown in the lovely 'bell-shaped' graph above, we can see that **just over 95%** of the data is within two standard deviations either side of the mean. We can make use of this when comparing data.

± 2 Standard deviations Worked Example 1. Lions



You have found the following ages of lions in two different zoos. The lions were randomly selected from all the lions in each zoo.

Age of Lions at Bristol Zoo (months)	Age of Lions at London Zoo (months)
36	46
31	50
35	48
24	49
21	51
47	49

The standard deviation is calculated using the formula:

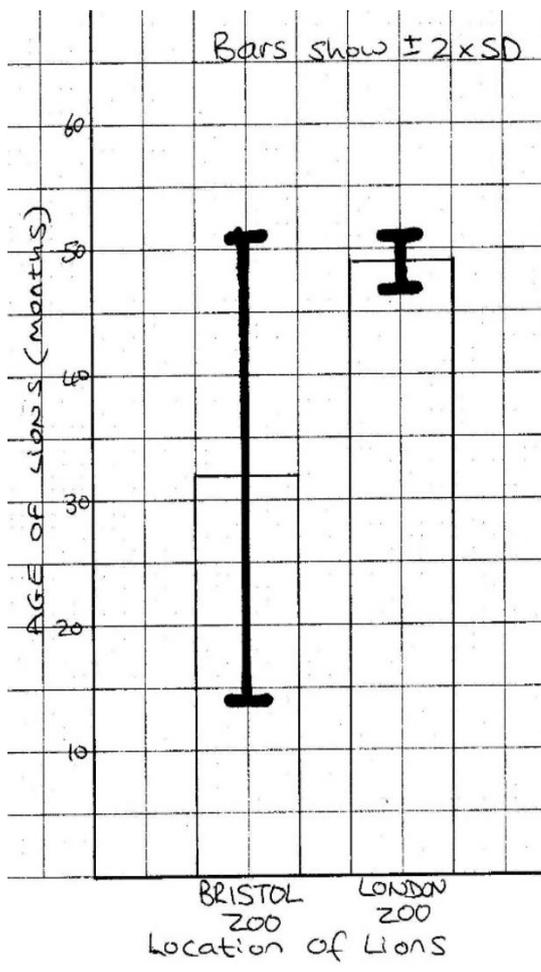
$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

The best way to show these calculations is in do this is in a table.

	Bristol Zoo	London Zoo
mean	32.3	48.8
SD	9.3	1.7
2 x SD	18.6	2.4
Mean + (2 x SD)	50.9	51.2
Mean - (2 x SD)	13.7	46.4

Describing the results

We can draw a bar chart of the mean and plot the ± 2 Standard deviations from the mean and look at the overlap of the bars.



Describing the data

There is an overlap in the (± 2 SD) bars.
 This indicates that the differences in the means (the age of the Lions at Bristol zoo and London zoo) are **likely** to be due to chance.

You now need to use the worked example to compare the data for catalase enzyme activity at 35°C and 45°C. Describe the data.

Question 1 Enzyme activity

The data shows catalase activity at two temperatures. The experimenter carried out 6 repeats.

Catalase enzyme activity (s-1)	
35 (°C)	45 (°C)
117	95
156	155
124	131
128	160
139	145
143	98

1 calculate the mean and standard deviation.

	Catalase enzyme activity (s-1)	
	35 (°C)	45 (°C)
mean		
SD		
2 x SD		
Mean + (2 x SD)		
Mean - (2 x SD)		

Describing the results

2 plot the bar chart on graph paper and draw on the 2 x SD bars.

3 describe the data by completing the box below.

Describing the data

There is **an / no** overlap in the (± 2 SD) bars.

This indicates that the differences in the means (.....) is **unlikely/likely** to be due to chance.