Biology investigation to investigate how selected variable affects the rate of fermentation in yeast

Planning

I think that the easiest way to measure the rate of fermentation in yeast is to measure a waste product of respiration. When the yeast is unable to respire aerobically it respires anaerobically producing alcohol and carbon dioxide. I could measure either of these two things to measure the rate of reaction. To measure the volume carbon dioxide is easier. I will do this by counting the number of bubbles of carbon dioxide through a wine trap with water in it. This gives a good indication of how fast fermentation is occurring.

I predict that as the temperature rises so will the rate of reaction in the yeast and therefore the volume of carbon dioxide produced. However when temperatures exceed 40°C the rate of reaction will start to decline and beyond 70°C the reaction will cease and if temperatures are significantly below 40°C (20°C) there will be very little or no reaction.

I am of this opinion because of the way enzymes are affected by temperature and because yeast contains enzymes. I know that enzymes have an optimum working temperature of about 40°C and that below this they fail to work effectively and above it they become denatured and fail to work. After about 70°C all of the enzymes will have become denatured and none will work.

There is just one variable in this experiment. I will change the temperature. The range of values that I have chosen is as follows:

25°C 35°C 40°C 55°C 70°C

It is important that I keep constant all other factors so that the experiment is a fair test. I will keep the same:

- The volume of the yeast suspension ($50cm^3$)
- 2g of yeast in $50cm^3$ of water (4%)
- 2g of sugar in $50cm^3$ of water (4%)
- The type of yeast used ("Saccharomyces")
- The same fermentation vessel
- Volume of water in wine trap (2ml)

The apparatus I shall use is as follows:
600cm³ beaker
250 cm³ beaker as water bath
400cm³ mixing plastic tripour
75 cm³ tripour for mixing sugar
50cm³ tripour for holding sugar
50cm³ tripour for holding yeast
Wine Trap
100cm³ Fermentation vessel with bung
Electronic Balance
50cm³ measuring cylinder
Funnel
2 x spoon
Bunsen Burner, tripod, gauze, insulation mat,
Kettle
Pipette dropper
Tap Water
Stirring Rod
Goggles
Insulation Material and Elastic Bands
2ml syringe
Pre-prepared results table

I will be using the following in my experiments:
Granulated sugar ('Silver Spoon')
Freeze-Dried Saccharomyces yeast ('Tesco')

My method will be as follows:

Step 1

Firstly it is important that the apparatus is easily available to hand and is well organised. Then, depending on the temperature you are testing, you can either put 250cm³ of cold, tap water or water from the kettle into the 600cm³ beaker. Then measure the temperature with a thermometer. You need to heat the water until it is above your required temperature, preferably about 15°C above. Do this using a Bunsen burner, tripod and gauze. Make sure you are wearing goggles at all times when working with a Bunsen burner.

Step 1b

Whilst the water is heating, measure out 2g of yeast and 2g of sugar and keep them dry, in a separate place. Add insulation material to water bath and secure with elastic bands.

Step 2

When the water has heated to the correct temperature (15°C above chosen temperature) pour 50cm³ into the 75cm³ tripour and 100cm³ into the water bath. Measure the temperature, which should have fallen to about 7°C above the required temperature. Place the remaining water back onto the heat and leave with the Bunsen burner on a low heat setting.

Step 3

Add the sugar, weighed previously, to the water that is in the 75cm³ tripour. Using the stirring rod, stir the solution until all of the sugar has been fully dissolved. Then pour this solution into the fermentation vessel.

Step 4

Using the funnel add the yeast to the fermentation vessel and replace bung. Next add 2ml of water to wine trap. Then with finger on the bung shake the vessel vigorously for about 40-60 seconds. After this, remove bung and as quickly as possible replace with wine trap. Immediately immerse the fermentation vessel complete with wine trap into the water bath.

Step 5

Using the thermometer check that the temperature of the water bath is exactly as desired. If it is too low then add hot water from the large beaker being heated by the Bunsen burner, using the pipette dropper. If it is too high then add cold water. Keep doing this over the course of 3 minutes, maintaining your desired temperature. During this time record the number of bubbles passing through the wine trap and record them in a pre-prepared results table. Repeat this at least twice for each temperature value that you have chosen and then calculate the average based upon the two readings. Record this in the table as well.

Obtaining Evidence

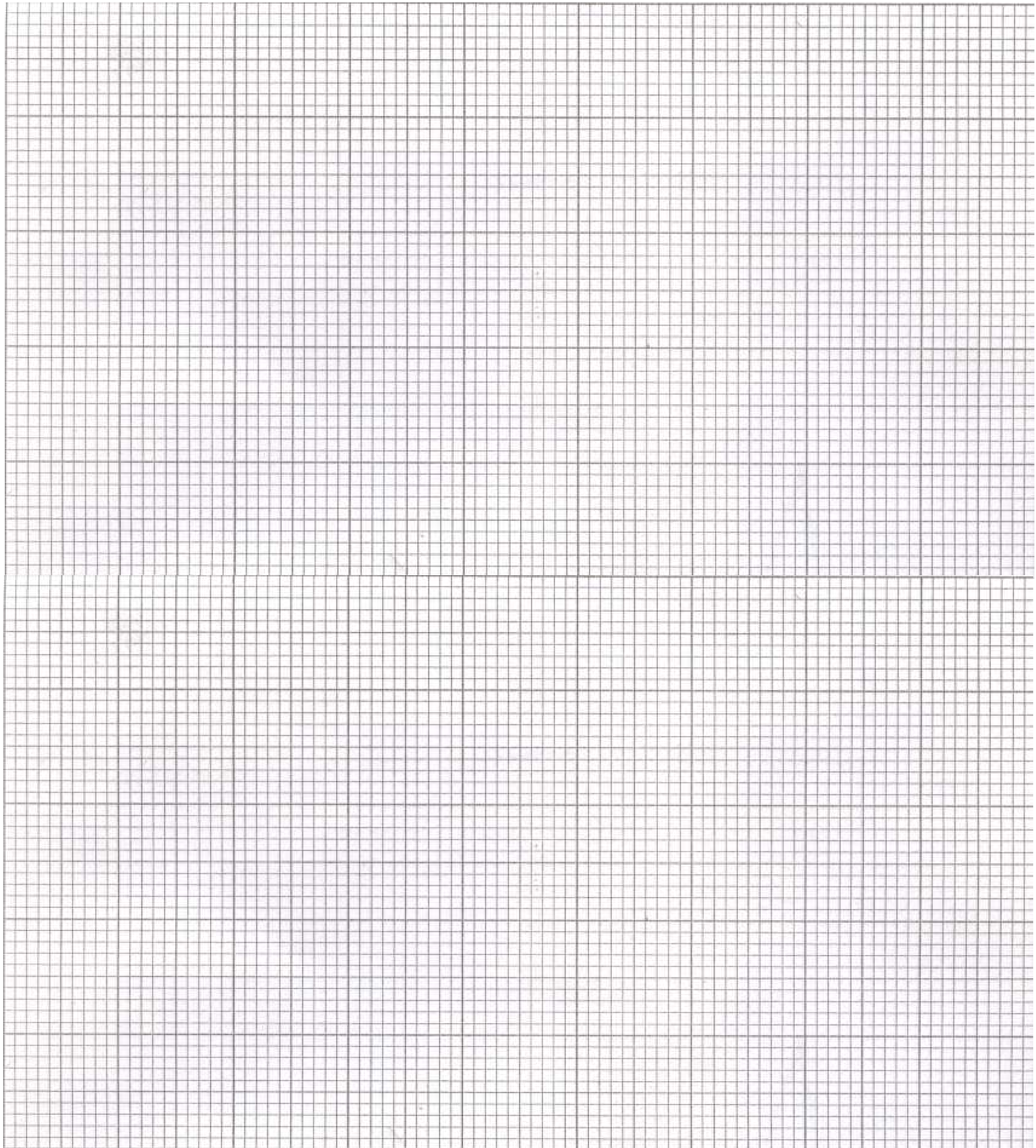
I use the above method to obtain my results. Using that method these are the results I obtained:

Values (°C)	Reading 1 (number of bubbles)	Reading 2 (number of bubbles)	Average (number of bubbles)
25	3	0	1.5
35	8	12	10
40	15	17	16
55	10	10	10
70	0	0	0

To make sure that the number of bubbles counted was accurate I disregarded the first few, especially at the higher temperatures. This is because heated air expands and causes bubbles of air, not carbon dioxide to pass through the wine trap.

Analysing Information

Graph:



The results in the graph above confirm my prediction. I said that I thought that the rate of fermentation would increase and then peak at 40°C and then start to decline until no bubbles would be seen at 70°C. This was proved to be the case. This is because of the way that enzymes within the yeast work to use the sugar and convert it into alcohol and carbon dioxide. Enzymes have an optimum temperature and this is about 40°C. Beyond this they fail to function correctly, and below it they do not have enough energy to collide with the sugar particles. This is because the active site of the enzyme changes shape and the substrate (a glucose particle) in this case no longer fits. This is called denaturing and reduces the rate of reaction significantly. The diagram below shows a normal enzyme and substrate and then a denatured enzyme and substrate.

Evaluation

I am pleased with the results that I obtained using the method that I have described. The results fit all of the scientific theory and my prediction. There were no obvious anomalies in my results. The results from repeat reading are slightly different in most cases but never by anything greater than 4. This does however show that the results could have been better. A 3rd repeat of the experiment would have been preferred enabling me to produce a more accurate average. Unfortunately I didn't have time.
There was one difficulty in counting the numbers of bubbles. As mentioned earlier the heated water which was added to the water bath caused the top of the fermentation vessel to heat rapidly and the air inside to expand forcing air through the wine trap producing bubbles. These had to be distinguished from carbon dioxide bubbles which were to be counted. Had I had more advanced equipment I would have liked to use a thermostat and some means of electronic heater to control the temperature of the water bath and produce more accurate readings. I had biased my suggested temperature values to be more concentrated around the 40°C mark but I think that it may be worth doing more in this

range perhaps trying to do it at 1°C intervals, from say 35°C - 45°C. This would be difficult however and would require more advanced equipment such as a thermostat and electric heater immersed within the water bath. With this level of accuracy it may be worth then using a gas measuring syringe to measure accurately the volume of carbon dioxide produced.