Useful Yeasts – Factors Affecting Respiration

Aim:

Part A: to identify the gas produced when yeast cells expire

Part B: to investigate the effect of different temperature ranges on the rate of respiration of yeast cells

Part C: to investigate the effect of increasing enzyme concentration on the rate of respiration of yeast cells

Hypothesis:

Part A: it is expected that when the yeast is breaking down the glucose there will be two gases produced; ethanol and carbon dioxide.

Part B: it is expected that the yeast respiration rate will increase as temperature increases, until enzyme begins to denature.

Part C: it is expected that the increasing the yeast concentration will cause the respiration reaction to increase until there is too much enzyme compared to substrate.

Materials:

(Part A as provided)

Parts B & C:

- Large beaker
- Three test tubes
- Three one hole stoppers with droppers inserted to fit the test tubes
- Glass stirring rod
- Thermometer
- 10 ml measuring cylinder
- Funnel
- Pencil
- Timer
- Masking tape
- Brass weights
- 20% glucose solution
- Dried yeast
- Teaspoon measurement
- Ice or kettle

Method:

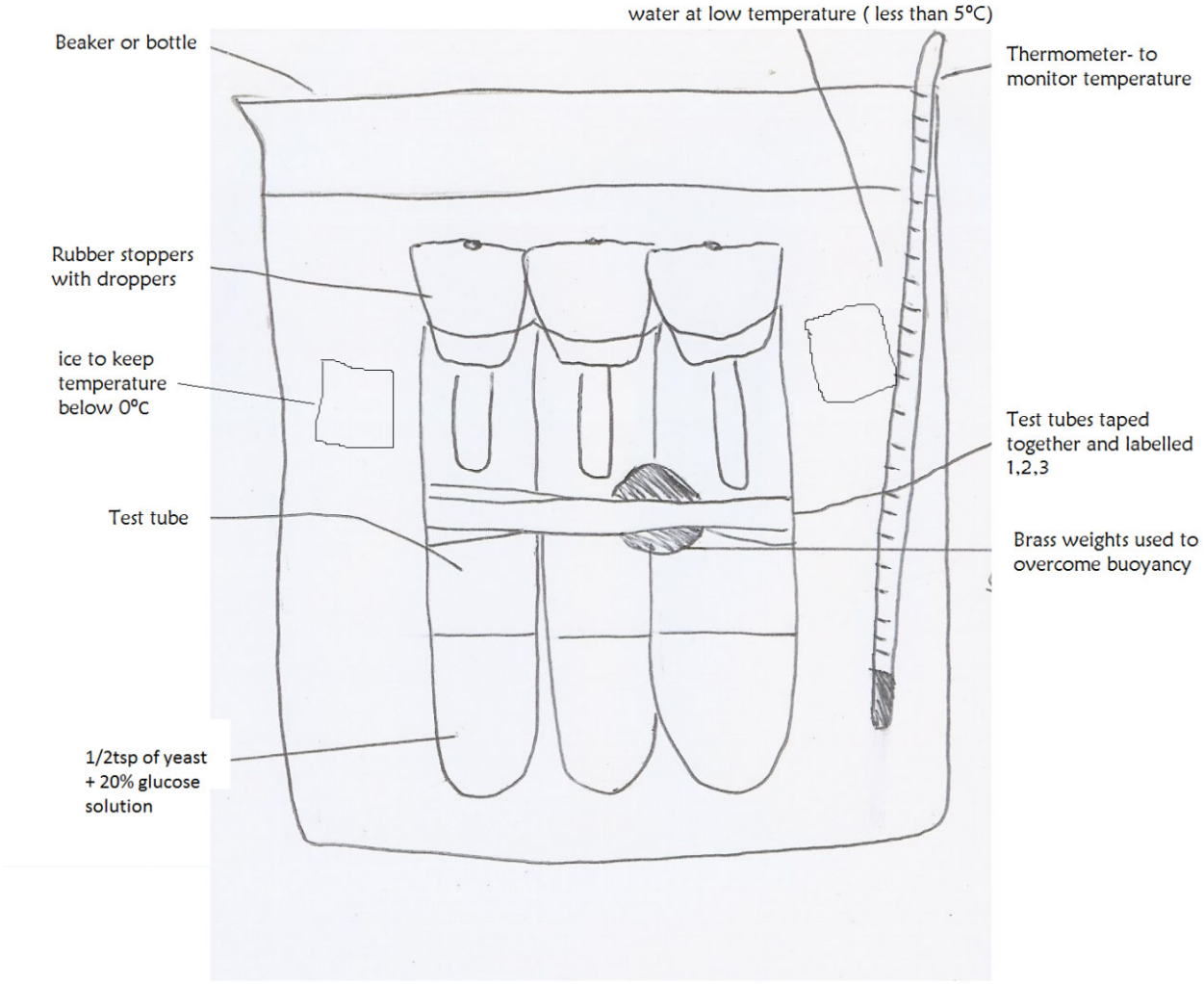
(Parts A & B as provided)

Part C:

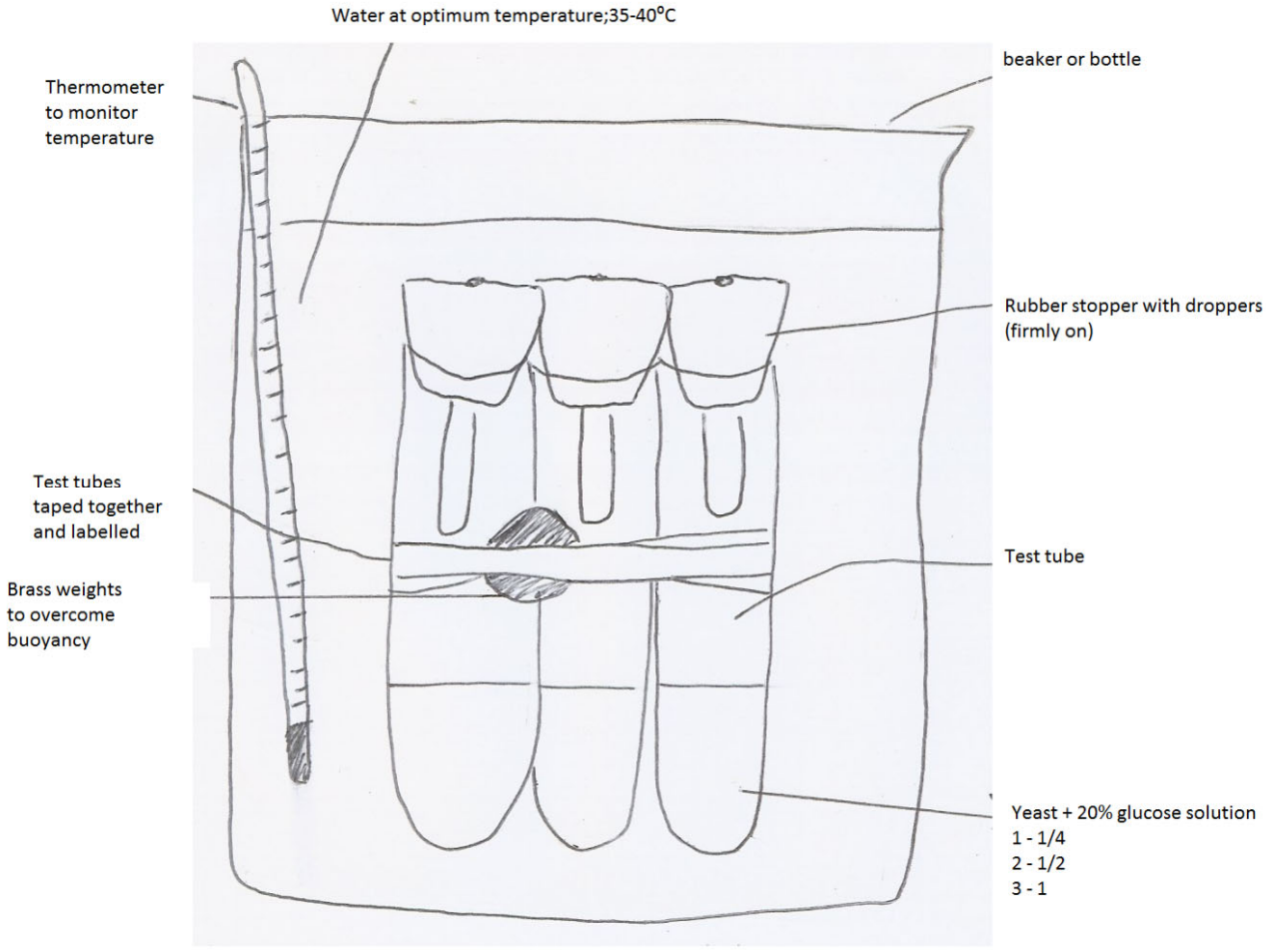
1. Collect the materials and begin to heat up the water to the optimum temperature: 35-40°C
2. In each group there will be 3 observers, 1 recorder and 1 temperature monitor
3. Label the three test tubes 1- ¼tsp concentration ,2- ½tsp concentration & 3-1tsp concentration
4. Add 12 ml of 20% glucose to each test tube
5. With masking tape, stick the test tubes together and add brass weights so the test tubes will remain submersed in the solution
6. Fill the large beaker with the water and maintain the temperature within the range of 35-40ºC, monitor temperature with a thermometer
7. When ready to begin; add the concentrations of yeast to each specific tube (test tube 1= ¼tsp concentration ,2= ½tsp concentration & 3=1tsp concentration)
8. Use the stirring rod to disperse the yeast in the glucose solution
9. Insert the rubber stoppers with droppers, making sure it is a firm fit.
10. Put the three test tubes into the water beaker, making sure they are completely submerged
11. Leave the tubes to acclimatise in the water for 3 minutes
12. The count the number of bubbles released every minute from each test tube for 10 minutes.
13. Record the data on a table
14. After the 10 minutes has passed, dispose of the solution and return equipment

Diagram of experimental setup:

Part B

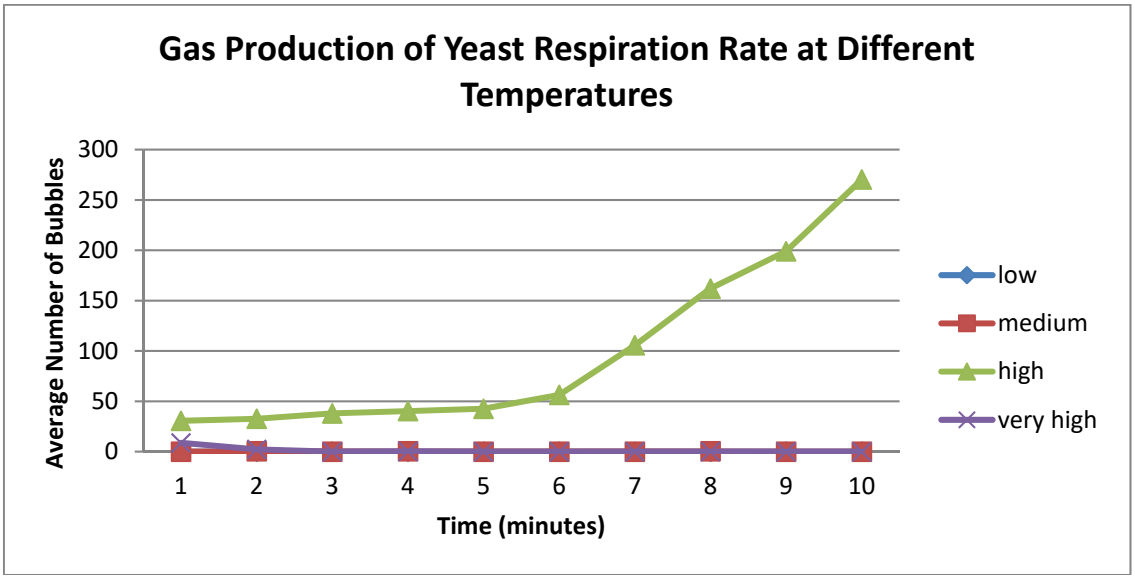


Part C


Water at optimum temperature;35-40°C
Thermometer to monitor temperature
beaker or bottle
Rubber stopper with droppers (firmly on)
Test tubes taped together and labelled
Test tube
Brass weights to overcome buoyancy
Yeast + 20% glucose solution
1 - 1/4
2 - 1/2
3 - 1

Part B: Gas production from yeast respiration at different temperatures

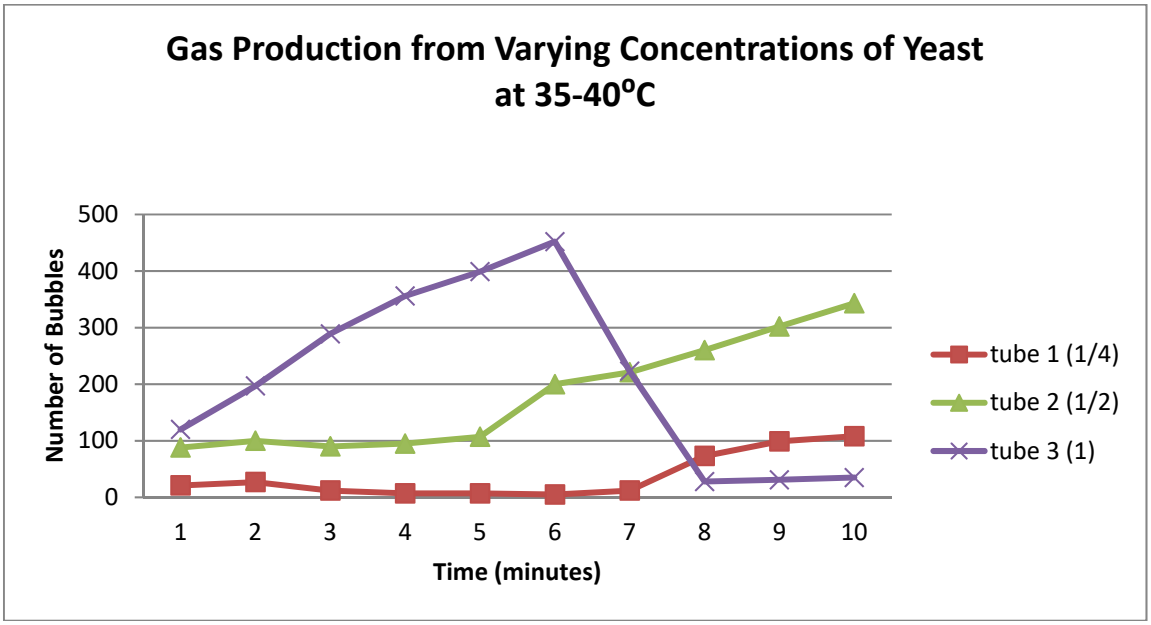
Time (minutes)	Average gas bubbling rate at low temperature (less than 5°C)	Average gas bubbling rate at medium temperature (15-20°C)	Average gas bubbling rate at high temp (35-40°C)	Average gas bubbling rate at very high temperature (+60°C)
0	-	-	-	-
1	-	-	30.6	8.7
2	-	0.33	32.6	2.3
3	-	-	38	-
4	-	0.33	40.3	0.3
5	-	-	42.6	-
6	-	-	56.3	-
7	-	-	105.6	-
8	-	0.33	162	-
9	-	-	199	-
10	-	-	270.3	-



At the low temperature it can be observed that the yeast respiration rate was unapparent and no bubbles were recorded. This suggests that the yeast enzymes did not function well at low temperatures due to decreased efficiency. At the medium temperature, there was 0.3 bubbles recorded at 2, 4 and 8 minutes. Although there was some gas produced, the respiration rate was still slow. At the high temperature the gas production rose steadily from 30.6 bubbles at one minute to 56.3 bubbles at 6 minutes, at 7 minutes the number of bubbles increased to 105.6 and then finished at 270.3 bubbles at 10 minutes. This occurred due to the higher temperature increasing the rate of the reaction, the extra heat energy made the molecules move faster. The faster the molecules moved the more often the substrate and enzymes came into contact which caused faster production of the product. But too much heat does damage the structure of the enzyme as shown with the very high heat. At 1, 2, and 4 minutes there was 8.7, 2.3 and 0.3 bubbles recorded accordingly then after the 4 minutes there was no further gas production observed. The very high temperature of 60 ºC caused the enzymes to become denatured; this is an irreversible change in the protein structure which means the enzyme can no longer bind to the substrate.

Part C: Gas production from varying concentrations of yeast at 35-40°C

Time (minutes)	Number of bubbles in tube 1	Number of bubbles in tube 2	Number of bubbles in tube 3
1	21	88	120
2	27	100	197
3	12	90	289
4	7	95	356
5	7	107	399
6	5	200	452
7	12	221	223
8	73	260	28
9	99	302	31
10	108	343	35



Test tube 1 which held the smallest concentration of yeast (¼tsp) had the slowest rate of reaction with the gas production gradually increasing from 21 bubbles at 1 minute to 108 bubbles at 10 minutes. Test tube 2 with ½tsp of yeast had a faster rate of reaction than tube 1 as the higher concentration of enzyme speed up the reaction rate without affecting the final amount of product produced. Test tube 3 held the largest concentration of yeast with 1tsp, the gas production escalated progressively until 6 minutes where it suddenly declined and at 8 minutes 28 bubbles were recorded. This was triggered by the solution becoming completely saturated at 6 minutes; there was too much enzyme concentration and not enough substrate to see the reaction through.

Difference between Part B & C

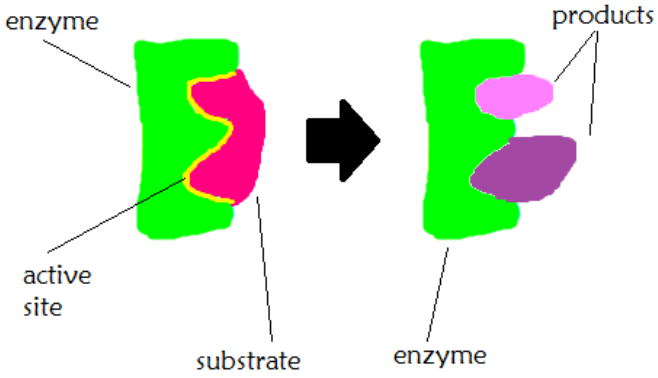
Part B was testing the effect of different temperatures on the one concentration (½tsp) of yeast whilst part C investigated the effect of different concentrations of yeast on the rate of respiration at the optimum temperature. In part B it was observed that the high temperature of 35-40ºC was the optimum temperature so this was then utilised in part C.

Discussion:

A catalyst is a substance that accelerates the rate of a chemical reaction, without actually being used up in the reaction. Enzymes are catalysts that are made of proteins; every enzyme is specific and has an active site – the region of an enzyme into which the substrate (what an enzyme binds to) molecule fits.

Within our body, functioning cells carry out many activities which includes manufacturing (synthesising) organic molecules, transforming energy, and breaking down and recycling unwanted substances. The chemical reactions involved in these processes are controlled by enzymes. Products are formed as a result of enzyme action. Enzymes can 'build things' or 'break things down'. An important role of enzymes is in the stomach where they aid with digestion. They are secreted into the gut to increase the rate of breakdown of food into molecules small enough to be absorbed across the membranes of the gut lining and into the body.

Diagram of Enzyme Function:



Without enzymes, the chemical reactions might still occur but at very slow speed that life would not exist. It is very important that the enzymes are not damaged. They can be damaged by influences such as temperature and acidity (pH). Changing the pH changes the shape of the enzyme and binding cannot occur and therefore most enzymes work best at particular pH values. All enzymes have an optimum temperature range and the reaction rate generally increases with temperature. A temperature that is too high can negatively affect the enzymes by irreversibly changing their protein structure also known as denaturation. Boiling in water is a typical way of denaturing enzymes; this is why some vegetables are blanched in boiling water before being frozen, to stop the vegetable deteriorating during storage. Factors that affect enzyme output are; the enzyme concentration, the substrate concentration and inhibitor molecules.

In cells, fermentation is the breakdown of glucose that follows glycolysis when there is no oxygen present, this produces either lactic acid (most animals) or alcohol (in most plants and microorganisms). It is a chemical change brought on by the action of microscopic yeast, moulds and bacteria. The souring of milk, the rising of dough and the conversion of sugar to alcohol are all examples of fermentation. Alcohol (beer) is made by taking a grain, such as barley, wheat, or rye, germinating and drying it, and pulping it into a mash. This mash is then mixed with hot water, and some fermentation begins. After being further treated, the liquid is transferred to a fermentation vessel, where yeast is added to the mixture. This yeast “eats” the sugar present in the mash and converts it into carbon dioxide and alcohol. After a few weeks of fermentation and a further period of conditioning, the beer is ready to be filtered and consumed. This process can be represented by the following equation:

Yeast + Glucose → Alcohol (Ethanol) + Carbon Dioxide

During the time the Part A experiment was set-up, it was detected that yeast was breaking down the glucose as there was evidence that gases were produced. These two gases were carbon dioxide and ethanol identified by the lime water bubbling and turning a milky colour. The number of bubbles also hinted to how hard the enzymes were working which determined the speed of the reaction.

The bubbles can be used to measure activity or the rate of respiration as the bubbles signify that there is gas being produced as a result of the yeast reacting with the glucose, the more bubble coming out from the tube, the more gas being produced and therefore the faster reaction speed.

Evaluation:

One error could have been not maintaining the required temperature for the specific test. For example in part C where the optimum temperature of 35-40°C was needed to investigate the effect of different concentrations of yeast. If the temperature fell below then the enzyme efficiency would have decreased and if above the enzyme protein structure would have become permanently damaged. This would have had a detrimental effect on the results as the final recordings would have been either at 0 or falling rapidly. To minimise this error in the future, the temperature should be checked regularly by means of a thermometer. Another possible error could have been using the incorrect amount of 20% glucose solution, if there was too much this would have increased the amount of product produced. By accurately using a measuring cylinder and measuring 12mL this error can be prevented in the future.

Conclusion:

The aims were achieved; the type of gas produced when yeast cells expire was identified. The effect of different temperature ranges on the rate of respiration of yeast cells was investigated. Also the effect of different enzyme concentrations on the rate of yeast cell respiration was examined. The hypothesis for part A,B & C were supported and it was learnt that when subjected to a very high temperature the yeast enzyme begins to denature and the point of saturation is reached when there is too much enzyme and not enough substrate to see reaction through.