

INVESTIGATION INTO THE AFFECT OF TEMPERATURE ON YEAST ACTIVITY

AIM: To investigate the affect of temperature on yeast activity.

APPARATUS: 1 test tube, 1 boiling tube, rubber bung and delivery tube, test tube rack, 250ml beaker, tripod, gauze, Bunsen burner, stopwatch, 2 measuring syringes, thermometer, safety goggles, 250ml of glucose solution and 250ml of yeast solution.

DIAGRAM:

METHOD:

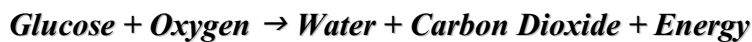
- 1) Set up the apparatus as shown in the diagram. Put on safety glasses and fill the 250ml beaker with water to slightly over half way. Also fill roughly $\frac{3}{4}$ of the test tube with water.
- 2) Measure out 10ml of glucose solution in one syringe and 10ml of yeast solution in the other. Add these two solutions together in the boiling tube.
- 3) Light the Bunsen burner and heat the water in the water bath to 15°C . When the water has reached temperature place the boiling tube into the beaker.
- 4) When the contents of the boiling tube have reached 15°C remove it from the beaker and fit the rubber bung and delivery tube. Place the loose end of the delivery tube into the test tube containing water.
- 5) Gently shake the glucose and yeast solution and start the stopwatch when the first bubble appears in the test tube. Time how many bubbles are released from the delivery tube in 1 minute.
- 6) Record the result and measure the temperature of the glucose and yeast solution to check it has remained at 15°C . If not, disconnect the rubber bung and delivery tube and place the boiling tube back into the beaker. Heat the glucose and yeast solution back up to temperature slowly.
- 7) Repeat the test from 4) again 4 times.
- 8) Repeat the test from 3) to 7) again, but heat the water in the beaker from 15°C to 25°C . Do the same for each of the temperatures 35°C , 40°C , 45°C , 50°C , 60°C and 65°C

SAFETY: Some safety measures must be adhered to during this experiment. Keep safety goggles on at all times during the experiment and ensure any loose clothing is removed or tucked in. Shake the glucose and yeast solution gently and carefully when performing the test so as not to spill any of the contents or drop any of the apparatus.

VARIABLES: The variables and constants in this investigation are as follows:

- The volume of glucose solution, which remains constant at 10ml.
- The time in which the amount of bubbles released from the delivery tube is counted. This remains constant at 1 minute for every experiment.
- The volume of yeast solution, which also remains constant at 10ml.
- The temperature of the sugar solution, which is recorded for each experiment and taken into account for any differences that it may cause to the results.
- The concentration of the glucose solution and yeast solution.
- The intensity that the glucose and yeast solution is shook at. This must remain constant at a gentle rate as a minor change in shaking intensity can significantly alter the rate at which bubbles are produced.
- The independent variable in this investigation is the temperature of the glucose and yeast solution. This input variable will vary in each experiment starting at a temperature of 15oC and increase by 10oC until 45oC is reached. I will also include 40oC in the investigation.
- The amount of bubbles, which is the outcome variable.

THEORY: Organisms obtain energy by *respiration*. Normally sugar (glucose) is broken down in the presence of oxygen to produce carbon dioxide and water:



For glucose to be broken down in this way oxygen is essential. Therefore this process is called *aerobic respiration*.

However, if there is no oxygen available the organism will either suffocate or use a similar process called *anaerobic respiration*. This involves sugar being broken down and energy being released without oxygen.

Yeast also respire anaerobically by converting sugar into *ethanol (alcohol)* and carbon dioxide gas:



This process is called **alcoholic fermentation**. The amount of energy produced in anaerobic respiration is significantly less than in aerobic. In aerobic respiration the sugar is broken down completely; in anaerobic respiration sugar is only partially broken down and there is a lot of energy still locked up in the ethanol. Burning some ethanol can show this – the energy in it is then released as heat. Though inefficient, alcoholic fermentation is a useful way of obtaining energy when oxygen is scarce. In this investigation, the yeast will be respiring anaerobically since it is not exposed to oxygen.

Fresh yeast has a similar appearance to putty. But it is really a fungus consisting of millions of tiny living cells. A number of these cells are **zymase** enzymes that use the glucose during anaerobic respiration to form energy and the other waste products. Being a typical enzyme, the zymase functions more effectively under different conditions. One of the factors that have an effect on the rate at which the zymase converts the glucose into carbon dioxide and water is **temperature**. This is the factor I am investigating.

Wild yeast grows on the surface of fruit, feeding on sugar. In the right conditions it multiplies rapidly by budding; a small bump protrudes from a parent cell, enlarges, matures, and detaches. A few yeasts reproduce by fission, the parent cell dividing into two equal cells. Many cells can be formed in a short time. Yeast has been used for centuries in making alcohol and bread.

PREDICTION: As explained above, temperature is one of the factors that affect the rate at which the zymase enzyme converts glucose into carbon dioxide and ethanol. Therefore to make a suitable prediction, we must consider the properties of enzymes.

Enzymes are **biological catalysts**. They speed up the chemical reactions that occur in living things. The following points list the five important properties of enzymes:

1. They are always proteins
2. They are specific in their reaction
3. They can be used over again
4. They are sensitive to pH
5. They are destroyed by heat – this is because enzymes, being proteins, are **denatured** by heat. Most enzymes stop working if the temperature rises above approximately 45°C.

Based on this and point 5. above, the zymase enzyme functions slowly at lower temperatures and faster at higher temperatures – this can be explained by the **collision theory**. At high temperatures the glucose molecules are moving more quickly. They collide with each other and the enzyme more frequently, causing more successful reacting collisions. The opposite is true at lower temperatures where the particles move slower and less successful collisions occur.

Therefore I predict that respiration will occur more quickly at higher temperatures and more bubbles will be counted as the temperature increases because carbon dioxide bubbles are formed at a faster rate. At roughly above 45°C little or no bubbles will be counted – this can be explained by the nature of the zymase enzyme. The enzyme will

reach an optimal functioning temperature, where the greatest amount of respiration occurs. This will be approximately 45°C. If this temperature is exceeded the bonds holding the enzyme together are broken, meaning that the enzyme can no longer function properly. At this point the enzyme becomes denatured.

A line graph can also be predicted to show what the results may resemble when plotted:

BIBLIOGRAPHY: I obtained information for this investigation from the following secondary sources:

- Biology For Life text book by M.B.V. Roberts
- Britannica.com web site
- Microsoft Word 2000 – word processing resource

RESULTS: The results of the investigation are shown in the table below. The average bubble count was calculated using the following equation:

$$(Test\ 1 + Test\ 2 + Test\ 3 + Test\ 4 + Test\ 5) / 5 = Average\ bubble\ count$$

<i>Temperature (°C)</i>	<i>Bubble Count (bubbles per minute)</i>					<i>Average</i>
	<i>Test 1</i>	<i>Test 2</i>	<i>Test 3</i>	<i>Test 4</i>	<i>Test 5</i>	
15	3	4	3	5	3	3.6
25	11	10	21	15	11	13.6
35	21	20	33	27	22	24.6
40	17	17	30	26	18	25.6
45	29	28	16	27	30	26
50	16	15	17	20	17	17
60	8	2	0	1	0	2.2
65	0	0	0	0	0	0

ANALYSIS: The results of my experiment show that higher temperatures up to a point somewhere between 45°C and 50°C, where all respiration stops, increase the rate of yeast respiration.

My results can be presented in simple line graphs, with temperature (°C) on the ‘x’ axis and number of carbon dioxide bubbles produced through respiration on the ‘y’ axis.

When arranged in the line graphs it becomes apparent that my results for the experiments, excluding test 5, are significantly inconsistent. In an attempt to counteract this I calculated the average bubble count. This provided a fairly precise curve of best fit and straight line. From the graphs I am able to find the following trends and patterns in my results:

- As the temperature increased the rate of yeast respiration increased, up to 45°C where a decrease in respiration began occur – this displays a direct positive correlation followed by a direct negative correlation.
- The rate of yeast respiration does not increase with temperature in a linear fashion. Instead a curve towards the ‘y’ axis (number of bubbles) is formed, meaning that the rate of respiration increases more rapidly at higher temperatures than at lower temperatures – this curve is called a ‘hyperbola’.

I am also able to use my graphs to estimate results:

For example, at 30°C I would expect the yeast to produce 16.5 bubbles through respiration in test 1, 15 bubbles in test 2, 27.25 bubbles in test 3, 21 in test 4 and 16 bubbles in test 5. For the average of all five experiments I would expect $(16.5 + 15 + 27.25 + 21 + 16) / 5 = \underline{19.15}$ bubbles to be produced, or between 19 and 20.5 from the average graph.

As the yeast respired it produced bubbles, which I counted during each individual experiment. The yeast respire to produce energy, which is used to maintain all its life processes, for example, growth, reproduction and digestion. The process is carried out in the yeast and glucose solution mix. The yeast to release energy, with carbon dioxide and ethanol produced as by-products, uses the glucose. This is shown in the word and symbol equations below:

Glucose → Ethanol + Carbon Dioxide + Energy



Therefore I was counting the bubbles of waste CO₂ produced.

The yeast is able to use the glucose to form energy and the other waste products through the use of an enzyme called ‘zymase’. Therefore it is the zymase enzyme that causes respiration to occur in the yeast and glucose solution. Being a typical enzyme, the zymase functions more effectively under different conditions. One of the factors that have an effect on the rate at which the zymase converts the glucose into carbon dioxide and water is temperature. The enzyme functions slowly at lower temperatures and faster at higher temperatures – this can be explained by the collision theory. At high temperatures the glucose molecules are moving more quickly. They collide with each other and the enzyme more frequently, causing more successful reacting collisions. The opposite is true

at lower temperatures where the particles move slower and less successful collisions occur.

Therefore respiration occurs more quickly at higher temperatures, explaining why more bubbles are counted as the temperature increases because carbon dioxide bubbles are formed at a faster rate. At 65°C no bubbles are counted – this can be explained by the nature of the zymase enzyme. The enzyme reaches an optimal functioning temperature, where the greatest amount of respiration occurs. This temperature is 45°C in my experiment. If this temperature is exceeded the bonds holding the enzyme together are broken, meaning that the enzyme can no longer function properly. At this point the enzyme is said to be denatured. This explains why no bubbles are formed at 65°C in my experiment as the zymase has become completely denatured at this point.

My results strongly agree with the prediction I made earlier in the investigation, as the rate of respiration increased with temperature up to a certain point, 45°C, where respiration no longer occurred. Also, the rate of respiration did not increase in a linear fashion, but increased more rapidly before reaching an optimum temperature of 45°C.

EVALUATION: The method I used in this investigation was simple, easy to repeat and of fairly high accuracy. However, I found counting the bubbles formed to measure respiration imprecise as is evident from the considerable fluctuation of my results. For example, at 45°C I counted 29, 28, 16, 27 and 30 bubbles for each test – a range of 14. I also believe that I obtained a wide range of results by using nine different temperatures, but I could have included 55°C to improve the accuracy of the curve.

I used 10ml syringes accurate to the nearest 0.1ml to measure the amount of yeast and glucose solution. A stopwatch accurate to the nearest 0.01 of a second was used to measure the 1-minute time intervals. I used a thermometer accurate to the nearest 1°C and used to measure the temperature of the yeast and glucose solutions. As stated before, the main inaccuracy in the experiment was met when counting bubbles to measure the rate of yeast respiration.

The results obtained in my experiment were not particularly consistent and several results stood out to be anomalous. For example, one obvious anomaly can be found at 45°C in test 3, where 16 bubbles were counted when compared to 29, 28, 27 and 30 in the other four tests. There is a possible reason for this anomaly – I may have shook the glucose and yeast solution too gently, causing a decrease in the amount gas bubbles produced. This may have occurred on several occasions and been the opposite in some cases – I may have shook the glucose and yeast solution too violently, causing an increase in the amount of gas bubbles produced.

Despite the several inconsistent results and the inaccurate way of measuring the amount of carbon dioxide gas produced, I would credit the method I used for being simple, safe and easy to repeat.

Two possible alterations that could be used to greatly improve my original method would include the following:

- Re-positioning the delivery tube into a measuring cylinder so that it could be used to collect the bubbles of gas. This would give an exact volume of gas produced by the respiring yeast. It would also reduce human errors caused by missing bubbles or counting extra bubbles – I found on a number of occasions that small bubbles were released from the delivery tube at the same time as the large and definite bubbles I had been counting.
- Using a much smaller temperature interval between each reading, such as 5°C, and a wider range of temperatures, such as 0°C – 70°C, would give more consistent results that could help to improve the accuracy of the experiment.

As explained above, the only factors hindering the reliability of my evidence were the method of counting bubbles to measure the rate of yeast respiration, the fairly large temperature boundaries and the narrow temperature range. These factors all combined to give a smaller amount of results than could be obtained for a successful investigation.

I also found a strange pattern in my results table that did not relate to the averages or the graphs. It showed a significant increase in the amount of bubbles produced on test 3 of each temperature, which rose by roughly 12 bubbles when compared to tests 1 and 2 that were quite similar. There is a possible reason for this pattern: after test 2 at each temperature the temperature of the yeast and glucose solution had fallen, so I reheated it back to temperature. Doing this must have re-acclimatised the yeast and caused it to produce bubbles at an increased rate. However, once the temperature fell again after reheating, lower bubble counts were obtained for tests 4 and 5, so averaging out the results.

The evidence I have obtained in this experiment is reliable enough to support the conclusion that the rate of yeast respiration increases with temperature up to 45°C, and then the zymase enzyme becomes denatured. However, I do not believe that the evidence is strong enough to support a numerical conclusion; such as if the temperature is doubled then the rate of respiration is doubled. More accurate experiments would need to be carried out before any reliable numerical conclusions could link the respiration rate of yeast and temperature.

A useful extension for this investigation would be to measure the rate of yeast respiration beginning from a temperature of 45°C and increase the temperature very slightly, for example, in 2°C or 3°C intervals, until respiration no longer occurred. This would find the approximate point where the zymase enzyme becomes denatured as well as the optimum functional temperature for the enzyme indicated by the highest respiration rate. Also, to further extend this investigation, I could examine another factor affecting yeast activity, such as the concentration of glucose or yeast solution.