Investigating the Factors Affecting Respiration in Yeast

Plan

In this experiment, I will be testing how temperature affects the rate of respiration in yeast. Respiration takes place when cells break down glucose to release energy using oxygen to help the process. Anaerobic respiration occurs when the cells don't use oxygen as one of the raw materials and alcohol is produced instead of water.

What we will be using to test the respiration is yeast. Yeast is a single celled (unicellular) fungus. It can respire without oxygen by converting sugar into ethanol (alcohol), energy and carbon dioxide gas, or with oxygen to produce water, energy and carbon dioxide.

The word equation for the anaerobic respiration is;

Glucose -> Ethanol + Energy + Carbon dioxide

The chemical equation is;

$$C_6H_{12}O_6 \rightarrow C_2H_5OH + \text{energy} + CO_2$$

The word equation for the aerobic respiration is;

Glucose + Oxygen -> Water + Energy + Carbon dioxide

The chemical equation is;

$$C_6H_{12}O_6 + O_2 \rightarrow H_2O + \text{energy} + CO_2$$

However our experiment will contain oxygen so therefore the equation that we will be using throughout our project would be the aerobic respiration equation.

Reasons Why We Are Doing This Experiment to Find the Rate of Respiration

Affecting Factors

The rate of respiration is a function of five different factors;

1. Temperature
2. pH
3. Types of food (sugar)
4. The concentration of yeast
5. The concentration of glucose

Temperature

The temperature affects the enzyme reaction. If the temperature is too low then the reaction speed will be very slow, as the temperature increases there will be an increase of kinetic energy, and therefore a greater rate of reactivity. The reaction will work best when it is at its optimum temperature, which is usually said to be between 40°C and 45 °C. If it is too high (above 50 degrees) then the enzymes will denature and the experiment won't work at all.

pH

A change in pH has a similar result. Enzymes are sensitive to the pH level; most work best in neutral conditions, not acidic or alkaline ones. An adjustment from a neutralto an acidic or alkaline state can destroy the enzymes, just as heat does.

Types of Sugar

There are many different types of sugars. While they may all be raw materials of respiration they could have different effects on the process. One sugar may increase the

rate compared to another which may make the respiring action go at slower than normal rate.

The Concentration of Yeast and Glucose

The concentration of yeast can also change how fast the reaction may go. If there is more yeast than other factors then it may influence the result rate to increase. If there is more sugar than the other factors it may also cause the experiment to speed up and create more energy from the glucose than it is meant to.

How will it be a Fair Test?

To keep it a fair test, it is necessary to keep constant, in all the tests, the other factors that are not being tested. This includes the concentration of the yeast and sugar and the type of sugar used.

To make sure the results are as correct and precise with the equipment I am using I will be measuring the temperature to one or two decimal places. The average temperature will be up to two decimal points long.

Measure of Rate

To measure the rate of the reaction, we could test how fast the raw materials (glucose) are used up, or we could test how fast the products (alcohol, energy and carbon dioxide) are made. We will be testing how fast the product, carbon dioxide, is produced.

To find out the rate of production of carbon dioxide, we first submerge a syringe containing the yeast and sugar mixture in a water bath. Next, we place a plastic tube on the nozzle of the syringe and do either of the following:

- The carbon dioxide produced will push out of the plastic tube on the nozzle of the syringe. We can count how many bubbles are produced within a given period of time.
- Place a second, smaller syringe that is filled with water onto the plastic tube. The carbon dioxide pr21oduced will push the water out the nozzle of the second smaller syringe. We can then measure the period of time within which the water is fully pushed out of the second syringe.

Independent and Dependent Variable

The independent variable (the factor that I change and is my input) is temperature. My dependent variable (the factor over which I have no direct influenceand is the output) will be the rate of carbon dioxide production. I will experiment with five different temperatures.

Reliable, Precise and Accurate

To make sure that the results are reliable, I will do each temperature test three times. If the results for the same temperature are similar, then we know that they are reliable. To keep the results precise, all the measurements will be measured to 3sf (three significant figures).

To confirm that the findings are accurate, I will check through my biology textbooks and see if they agree with my results.

My Prediction

The respiration rate will go quite slowly when it is at 30 degrees. It will, however, increase with the temperature, as they seem to be proportional to each other. I expect the rate of respiration will maximise at an optimum temperature of 40 degrees or perhaps slightly higher. However after the temperature rises to 50 degrees and over the enzymes shall denature because of too much heat. After the enzymes have denatured the experiment will be finished.

Apparatus Needed
Water Bath
Thermometer
Yeast suspension
Sugar (glucose solution)
Water
Syringe
Stopwatch
Kettle
Clamp
Oxygen

The yeast suspension contains 200cm^3 of water at 40^oC, 1 cube of sugar and 1 pack of bakers yeast.

The syringe will contain 5cm of air and 5cm of the solution. The solution will be a mix of 2.5cm of the glucose solution and 2.5cm of the yeast suspension.

Method

1st Part: Preliminaries

Preliminaries are what I need to do before I carry out the actual experiment. During the preliminaries, I will need to determine how much air is already present in the syringe when we place it in the water and how long this air takes to be expelled. This is necessary as the air already in the syringe will be pressured out and could be mistaken for CO_2 production. If I counted these, my results would be wrong.

I am using the first method for testing how much carbon dioxide is produced (counting the bubbles) within a specified period of time. Consequently, I will need to do this little experiment before I can start the main experiment.

For this, I will take the same size syringe and fill it with an amount of water that corresponds to the volume of the yeast mixture. I will then submerge it in the water bath at the same temperatures that I will be using in the experiment. I will then time how long it takes for all the bubbles to escape from the syringe and record the time. Subsequently, at the start of each experiment I will have to wait for that amount of time to pass before I begin to count the bubbles escaping from the tube.

From this I will be able to calculate how long each experiment will take as I need to decide how long each experiment will last after the water is equilibrated.

The time we need to wait for all the air to be pressured out of the syringe is 5 minutes 30 seconds

2cd Part: Main Experiment

I will conduct my experiment at five different temperatures and each of those I will test three times (to ensure the reliability of my experiment).

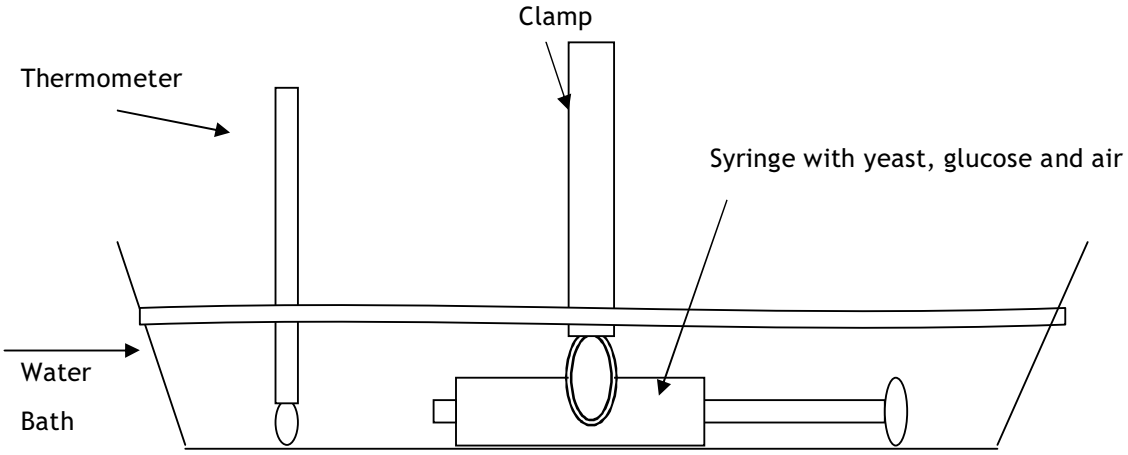
I will the range of temperatures from 30 – 70^oC, at 10^oC intervals. However, over 45^oC, I expect that the enzymes will denature, and it may be that I will not need to test higher than 50^oC.

At 30^oC, which is below what I believe may be the optimum temperature, we can see how fast yeast would respire if the temperature is not as high as it could be.

1. Collect all apparatus listed.

2. Heat the water to the temperature you are aiming for (30^{o}, $40^{o,}$ etc). Make sure it is as close to your intended temperature as possible.
3. Fill your syringe first with the glucose solution, then the yeast suspension. After you have the right amount pull the lever back enough so you have the amount of air you are going to use in the test.
4. When all this is ready attach a clamp to your syringe so it will be kept underneath the water and won't try to float up.
5. Record the temperature at which your water is when you place the syringe in the bath.
6. Start the stopwatch as soon as you put the syringe in the bath. Do not count the bubbles escaping the syringe until you have passed the air mark (preliminary test).
7. As soon as that time has passed start counting the bubbles coming out of the syringe, make sure to keep the stopwatch going– you need to know when your time is up.
8. Keep counting the bubbles until your set time is up.
9. Record the end temperature before anything.
10. Clean your equipment up and fill out the result table for that test.
11. If you are doing another test at the same temperature then you are able to keep the same yeast and sugar mixture. Repeat the same method from step 5 onwards. Make sure that the temperature is as close to the intended degree as possible before you begin the next test.

Diagram of the apparatus



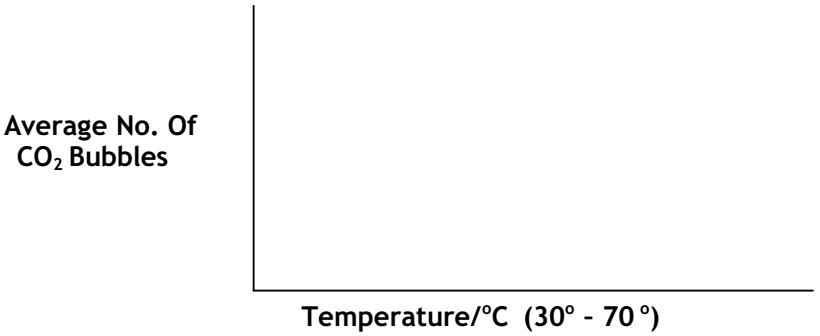
Example of Result Table (One table for each Temperature)

Test	Period Of Time**	Intended temperature	Actual temp. at beginning	Actual temp. at end	Average temp.*	No. of Bubbles produced

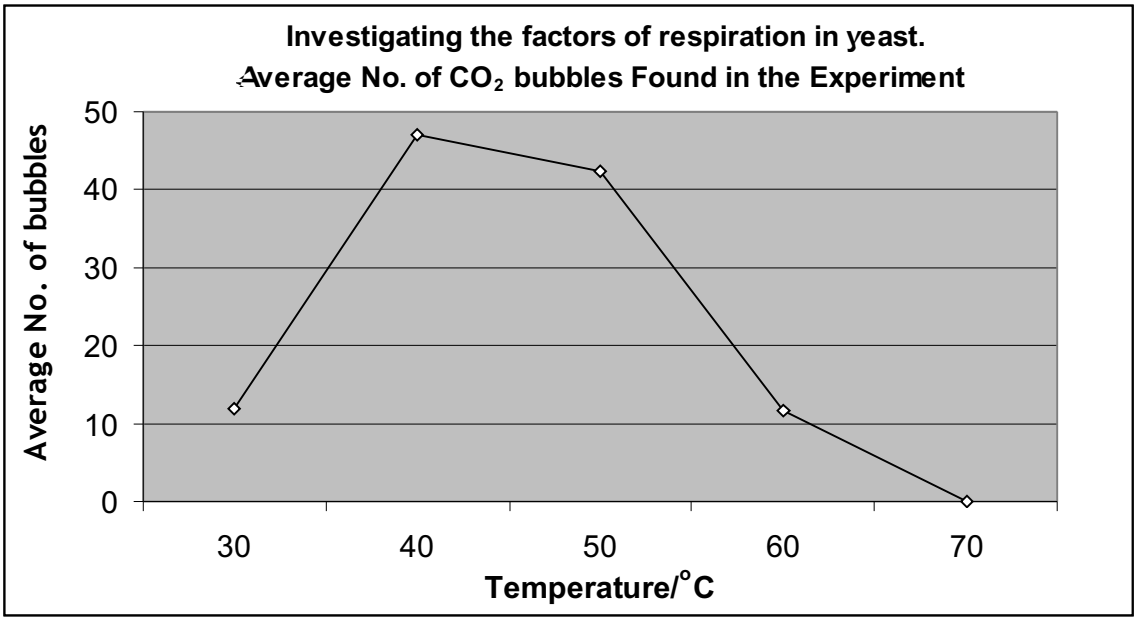
* to find the average temperature I will add the beginning temperature and the ending temperature together then divide the total by two and that will be the average.

** The period of time is the time after the air has been pressured out of the syringe.

Example of Graph Showing the Results,
(The graph will have all the temperatures and their average amount of bubbles)



Analysing and Considering Evidence



Looking at this graph it is very obvious that the optimum temperature of this experiment is 40°C which, according to the textbooks, is the usual result for the question of which temperature is the best for respiration. However we can see that instead of the enzymes denaturing shortly after 40°C they were still working very well at the 50°C range and only started to denature at 60°C and higher. We knew the enzymes had definitely denatured at 70°C as no bubbles were produced in that experiment as you can clearly see on the graph.

Prediction Vs Real Findings
My prediction was that the reaction rate would be quite slow at 30°C and it would then increase in speed as the temperature did until slightly higher over 40 degrees. I then said that once at 50°C the enzymes would denature because the water bath would be too hot for them to work any longer.

If you glance at the graph above you can see that the beginning of the experiment went how I predicted it would. The number of bubbles at 30°C is quite low, but once the temperature rises to 40°C there is a huge amount of bubbles. However I thought that after the temperature rose to 50°C the reaction speed would plummet suddenly because the enzymes would be dead. This was not the case though; the enzymes were still working well at 50°C and the amount of bubbles that were produced were the second largest amount in the experiment. The enzymes, though they were denaturing slowly, were still producing CO_2 at 60°C also. At 70°C nonetheless the enzymes had definitely denatured as no bubbles were produced at all.
Therefore my prediction was right for the lower temperatures but not fully right for the warmer temperatures.

Why the Evidence Did Not Support My Prediction
The evidence that was different to my prediction was at what point the enzymes would stop working. I said they would at 50°C, in actual fact they fully stopped working at 70°C. There are a few different reasons why this could be.
Reasons

1. I simply guessed the wrong temperature for when enzymes denature.
2. The glucose solution and the air inside the syringe could have affected the rate and helped the enzymes work at higher temperatures.
3. There may have been a mistake made at the start of one of the warmer temperatures experiments.
4. Although the intended temperature for the experiment was 60°C perhaps the actual temperature wasn't, or perhaps it was at the beginning but the temperature dropped so fast that the heat didn't have enough time to kill the enzymes.

Yes or No

1. This may be true, however my prediction for the temperature at which the enzymes denature was not just a random guess. I read through three textbooks and talked to my teacher about some of these details. They all agreed on that point – the enzymes usually denature when the temperature reaches 50°C and over.
2. The glucose solution was at room temperature when it was sucked into the syringe. This may have helped cool the yeast solution when it was put into the bath slightly. The air may have insulated the yeast and glucose mix from the water, helping cool them also, not only that the syringe which was made out of plastic did not get hot easily. Possibly it was doing the same thing as the air and also insulating the mixture that was inside from the hot water. Another way the yeast could have survived until 60°C is by somehow creating a favourable environment for itself that it was able to exist in for awhile.
3. This could have happen quite easily, except for the fact that I knew that this could occur without difficulty so I tried to do everything exactly as I had been told to on the first day, and how my method says to.
4. The intended temperature was 60°C of course and if you look at the results table you can see that the actual temperatures at the beginning were only two degrees off at the most. Therefore we know that that part of the experiment was not wrong. Looking at the temperatures at the end we can see that the first part of the experiment at 60°C ended at 45°C but the others ended in the lower 50°C. The reason behind the first part ending at such a low temperature is because that experiment went on for twelve minutes, and at such a high temperature the water cools very quickly. The second and third temperatures lowered quickly because of that reason too, but not because of the long period of time – these tests only took six minutes thirty. Because the temperature has dropped low enough to reach 50°C the enzymes would be able to respire quite well. However because the temperature was high at first some of the enzymes would have not worked well so that is why the amount of bubbles there is not as high as the amount of bubbles in the 50°C experiment.

Evaluating

Method and the problems that occurred

The method that I thought of and then used proved to give me reliable results in all of the tests but one. As I did each part of the experiment three times each and found similar results in them all I know that they can be counted on to be correct. However there was one part of one of the tests that gave me very erratic results. The temperature that I was testing was 40°C which is said to be the optimum temperature for yeast to respire. While this may be true the first test I did at this temperature showed me that the yeast had only produced two bubbles after six minutes and a half. Looking at the graph that shows us the average amount of bubbles found at each temperature we can see that what is said about 40°C is true. In spite of this I had to restart this test because of the first reading that I took, to find out whether the experiment was incorrect or I had done something wrong.

After I had repeated this fragment of the project and saw that many bubbles were produced I knew I had done something wrong when I prepared the syringe, however I do not know what I did wrong though I suspect that I might not have sucked any air in and therefore the respiration would be done anaerobicly , not aerobically. This means that not as much energy would be produced and therefore not as much CO_2 would be generated, so not many bubbles would come out of the syringe.

Evidence Means?
The evidence that I have collected shows me that the optimum temperature is 40°C as the textbooks have said, the enzymes have denatured not at 50 °C as we believed but at 70 °C. We can see that the yeast respired at 30 °C quite well which was quite expected. However if I had done three tests at a lower temperature (20 °C) than I may have found out at what temperature the enzymes have not got enough heat to respire.