Fermentation project

Aim

I am trying to find out what effects respiration in yeast.

Introduction

Yeast is any of a number of microscopic one-celled fungi used for their ability to ferment carbohydrates in various substances. Yeasts are extensive in nature, occurring in the soil and in plants. Most cultivated yeasts belong to the species Saccharomyces; those known as brewers yeast are strains of S. cerevisiae.
Yeasts have been used since the early years for the making of beer, wines and bread and today are used furthermore in industrial processes. Pure yeast cultures are grown in a medium of sugars, nitrogen sources, minerals and water. The final product may take the form of dried yeast cells. In the making of wines and beers, the fermented medium is the desired product and the yeast itself is discarded or used to feed animals.

Anaerobic respiration

Because yeast respires in anaerobic way fermentation can take place. Anaerobic respiration is respiration without the presence of oxygen.
The first step consists of breaking down glucose into pyruvic acid and the end product (in yeast respiration) will be ethyl alcohol.

Theory of fermentation

Fermentation is the chemical changes in organic substances produced by the action of enzymes. In alcoholic fermentation, the action of zymase (the enzyme) secreted by yeast allows anaerobic respiration to take place, converts simple sugars such as glucose and fructose into ethyl alcohol (ethanol) and carbon dioxide. It controls the speed of respiration (fermentation). The equation for this reaction is:

Glucose $\longrightarrow$ ethanol + carbon dioxide + energy
$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + (210\ kj)$

Possible Variables

The possible variables I have decided would be possible for my experiment are:

- Change in temperature – testing whether the heat in which the yeast is kept at during the experiment affects the rate of respiration.
- Amount of yeast – testing whether yeast respires faster when there is more or less.
- Amount of water – testing whether the quantity of water added to the yeast affects the rate of respiration.
- Amount of glucose – testing whether amount of glucose affects how quickly yeast respires.
- Light intensity – This would not be a sufficient variable as light does not affect respiration.
- p.H - by adding acid or alkali to the yeast to test whether it speeds up or slows down the reaction. Most enzymes work best at neutral conditions.

These are the variables I have decided on:
I have chosen to use temperature for my independent variable. The reason for my choice is that I know that enzymes are affected by temperature (as we increase the temperature we increase the rate of reaction).

Variable	Value or range	How measured
Dependant variable: Time		
Number of bubbles to be produced in a certain time	Measured: 10 minutes – the first 5 minutes will not be recorded as this is the time when the co that is already in the test tube is being released.	Digital stopwatch.
Independent variable: Temperature		
Temperature for 10 minutes of fermentation.	Range: 25 to 50 in steps of 5. Number of results: Repeats: each temperature will be repeated twice.	Thermometer.
Control Variables: These will be kept the same to keep the experiment a fair test.		
Amount of yeast and glucose solution	30ml	Measuring cylinder
Time	10 minutes	Stop watch

Prediction

I envisage that as the temperature of the water, in which the yeast is kept at increases, the rate of reaction will increase and more carbon dioxide will be given off in a certain time period.
As the yeast is heated, the particles gain more energy and move around more quickly. As they travel faster there are more collisions in a certain time. Therefore reactions get faster as you raise the temperature. Furthermore some colliding particles just rebound off each other. They don't collide hard enough for a reaction to occur, as they do not have enough energy. However at a high temperature, the particles are moving faster. They have a collision at a faster rate resulting in more collisions producing a reaction. I predict that although an increase in temperature may accelerate a reaction, enzymes are unstable when heated and so the amount of carbon dioxide (rate of reaction) will not be proportional to the temperature. E.g. doubling the temperature won't double the speed of reaction. It will not be that accurate.

Enzymes are large molecules made inside cells. When a substrate molecule bumps into a molecule of the right enzyme, it fits into a depression on the surface. This depression is called the active site. Anything that helps substrates to come into contact with the right enzymes will make enzyme-controlled reactions go faster (as in the reaction in yeast). This means that raising the temperature will increase the random movements of the molecules and increase the chances of substrate and enzyme colliding. Conversely enzymes will still allow respiration at low temperatures, as they are very efficient. Minute quantities of an enzyme can accomplish at low temperatures what would require violent reagents and high temperatures

by ordinary chemical means. As a catalyst cannot be used up, the rate of bubbles will be released at a constant speed. The rate at which the bubbles carbon dioxide is produced (how fast the bubbles emerge) will not alter with time.

However enzymes are proteins and become denatured if the temperature becomes too high. The active site changes shape and the substrate will no longer fit. Most enzymes stop working at around 45 C. This will mean that the rate of respiration will increase as the temperature gets higher until a certain temperature (around 45 C). I predict the enzyme will then become denatured and respiration will not occur. No CO will be produced.

Method

Before the experiment, a set of control results need to be obtained to compare against these will be carried out in the same way as the final experiment only using water in place of yeast.

Firstly a yeast suspension needs to be prepared. This can be achieved by pouring a packet of yeast into around 500ml of water at 20 adding glucose and stirring until the granules are dispersed. The solution will become cloudy and frothing will occur. This is the carbon dioxide. The suspension will then be used to achieve the following procedure.

1. Prepare a beaker of water at 50. To keep the temperature and prevent heat loss, wrap tin foil around the beaker.
2. Taking 30ml of solution from the prepared yeast suspension using a pipette and measuring cylinder, add to a test tube and place in the beaker.
3. Wait until yeast is the same temperature as the water before adding the bung and glass rod.
4. Using the pipette, add a few drops of water to the glass rod. This will allow you to be able to see when CO is escaping.
5. Start the stopwatch to time 10 minutes. Bubbles of CO will be released. Do not start counting the bubbles however until the first 5 minutes are up. This is to allow any CO that is already in the tube time to escape and so not effect the results.
6. Record the results after 10 minutes and repeat using fresh yeast with the water at the other temperatures.

Safety

When carrying out any experiment, certain safety procedures need to be carried out. Safety goggles maybe worn to protect the yeast solution from going into the eyes however in the unlikely circumstance it would do little harm anyway. However, when using hot water, care must be taken to avoid spillages resulting in scolds. Keep bags well away from the working area to avoid trips and if water is spilt, mop up immediately to prevent further accidents. Check glass beakers for any chips or cracks and be careful not to smash the beaker. If the beaker does smash, be cautious when sweeping up the pieces.

Results

Control results

Independent Variable Temperature C	Dependant Variable Number of bubbles in 10 minutes
50	18
45	11
40	9
35	9
30	5
25	1

Final results

Independent Variable Temperature C	Dependant Variable Number of bubbles in 10 minutes		Average dependant variable.
	Test 1	Test 2	
50	21	24	22.5
45	42 (78)*	40	41
40	35	36	35.5
35	16	18	17
30	6	7	6.5
25	5	4	4.5

*78 was an anomalous result occurring because the table was knocked resulting in a rush of bubbles. This result was retested and not used in the average.

Observations

I noticed that as the yeast fermented more froth was produced in the yeast solution. As the temperature increased, the froth was being produced faster.

Conclusion

The trend of the graph shows that *y* increases at an increasing rate.
The curve goes back down again meaning *y* has a maximum value. The interpretation of the graph is that the number bubbles produced increases as the temperature rises and increase at an increasing rate. The highest point in the graph is the optimum temperature at which yeast will respire at. However, when the graph slopes back down, this indicates the point at which the number of bubbles will not increase even if the temperature is increased. As the graph curves down, the rate of reaction decreases with time.
This is the point at which the enzymes start to become denatured (50). The enzyme zymase therefore denatures at a higher temperature than the average for most enzymes. This proves my prediction matches my results, although the denaturing temperature is higher. It is hard if not impossible to get an equation from the graph. This is because enzymes are unstable when heated.

Evaluation

The accuracy of my results is determined by the measurements of my variables. It was quite hard to keep the temperature constant throughout each test. The tin foil helped but the temperature sometimes dropped 1 or 2 degrees. To solve this, better insulation methods could be used such as more tinfoil or a layer of polystyrene as well. Then there was the accuracy in the amount of carbon dioxide being released. It was quite simple to count the number of bubbles, however each bubble was not the same size making it impossible to know the actual volume of carbon dioxide being released. To solve this, a more complicated experiment would need to be carried out using a piece of apparatus called the Ganongs respirometer. This is a u shape monometer with a tap to equalize the pressure. The tap is then closed and co pushes a salt solution through the tube. Depending on how much the solution has moved, the amount of co can be recorded. This allows the quantity of co in cm to be recorded giving more accurate results. My results are reliable as for both tests, similar results were achieved and the conclusion fitted both sets of results. However my conclusion is only valid to the range of values I have investigated. To get more accurate results I would use smaller steps between each temperature. I only know that the temperature of denaturing is between 45 and 50 C plus the enzyme isn't full denatured at 50 . Some bubbles are still being produced. I would have to extend my range to see what happens when the temperature is increased further.