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GCSE C

Investigating respiration in aged yeast

Planning

AIM: I want to investigate respiration in aged yeast.

Prediction

I predict that the older the yeast, the smaller amount of carbon dioxide is given off. Yeast (*Saccharomyces cerevisiae)* is a single celled micro-organism that belongs to the fungus group of plants, lives on the surface of fruits and feeds with sugar. It multiplies very fast by budding- each cell "pinches off" new ones and a large number can be formed in a short time. Yeast cells contain enzymes, this explains why the fermentation is taking place
Living yeast cells cause fermentation in the absence of air, converting sugar (glucose) to ethanol and CO_2 . The respiration in absence of air is called anaerobic respiration (also known as fermentation). Anaerobic respiration is the breakdown of carbohydrates to release energy, without the use of oxygen.
"Typical products of fermentation depend upon the substrate but can include organic acids (lactic acid, acetic acid), alcohols (ethanol, methanol, butanol), ketones (acetone) and gases (H_2 and CO_2)"
As yeast ferments glucose to ethanol, carbon dioxide is given off.
Glucose→ alcohol + carbon dioxide↑ + energy
At the beginning of the fermentation a bigger quantity of carbon dioxide is given off.
Left for several days in a warm place the alcohol can be distilled off.

Apparatus

- Conical flask with warm water- this is my water bath 250 ml
- Test tube containing the glucose and yeast solution
- Rubber cork connected to a plastic tube, that covers the test tube
- Thermometer
- Large plastic beaker
- Measurement cylinder 50 cm^3
- Scale
- Stop clock
- Syringe 10ml

Method

My preliminary experiment worked very well, after I changed some parts of the apparatus and the temperature of the water bath. From my preliminary experiment I learnt that yeast works better at higher temperature; I tried the experiment at 21˚C and the yeast didn't react, then I increased the temperature in the water bath to 39˚C and it started reacting, releasing carbon dioxide.
I also changed the conical flask- for the water bath- with a bigger one, to be able to keep the temperature constant for longer time.

- Measure with a syringe10 ml yeast dissolved in water solution
- Put the content in a test tube
- Weigh 3g of glucose and I add it to the yeast in the test tube
- Stir it well so the glucose dissolves completely
- Put the test tube in a water bath in the conical flask
- Cover the test tube with a rubber cork that is connected with a plastic tube
- Put the plastic tube in a measurement cylinder of 50 cm^3 that is full with water
- Put the measurement cylinder, upside down, in a large plastic beaker with water and make sure that there is no air in the measurement cylinder
- Check the plastic tube inside the cylinder and make sure that is deep enough, so no carbon dioxide escapes in the plastic beaker
- Start the stop-clock and register the time
- Register the water bath temperature and keep it constant by adding warm water if the temperature drops
- Read at the bottom of the meniscus to find out the volume of carbon dioxide released

To be able to collect valid data I'll measure everything very well and I'll be careful not to make mistakes; if I suspect that something is not exactly the amount needed, or the temperature required, I'll check again and make all the adjustments necessary.
I'll record the time when the yeast starts reacting very precise, with the stop watch, writing down the minute and the second, as well as the other intermediate readings at: 0.50 cm^3, 2 cm^3, 5 cm^3 8 cm^3, 9 cm^3 and 10 cm^3. I chose these intermediate readings because it will be easier to register the time and at the beginning of the experiment the amount of carbon dioxide released is slower than at the end, so I can see better the difference in the amount of carbon dioxide released on minute.
To make my investigation a fair test I'll use six different yeast solutions of different ages and investigate the amount of carbon dioxide released in time, record and analyse data; put the results in graphs and see if they fit the predicted trend.

Risk assessment

The apparatus and the method is relatively safe, but the only area of concern is that the most parts of the apparatus are made of glass, so I should be careful not to break any, or if this happens to remove the broken glasses with caution.

Variables

I will measure and record the following variables:
Variable to change: Age of yeast – I'll use different ages of yeast solutions every time. I'll prepare the solution a day before the experiment at different hours, so the yeast will have different ages; after I prepare the solution I'll put it in a flask and write on it the hour I prepared it, so I can calculate its age when I start the experiment.
Constant: I will use the same type and amount of the following:

- Type of sugar – glucose
- Concentration of sugar solution- 30%
- Volume of yeast solution - 10 cm^3
- Concentration of yeast – 10%
- Temperature- 39˚C

To keep these constant I'll use a high accuracy scale to measure the amount of glucose; use a syringe to measure the volume of yeast solution used; I'll put a thermometer in the conical flask and add more hot water to my water bath all the time, to keep the temperature constant.
I may not be able to control the test tube, if it has a small hole that may allow small amount of carbon dioxide to escape through it or the connection with the cork is not very firm.

Obtaining evidence

I used only one type of sugar: Glucose.
I prepared the yeast solution a day before the experiment and I arranged the apparatus.
I recorded all the data proposed as shown in the table:

Age of yeast (h:min)	Concentration of sugar solution (%)	Volume of yeast (cm^3)	Concentration of yeast (%)	Temperature (˚C)	Final time (min:sec)	Volume of CO_2 (cm^3)
20:30	30	10	10	39	53:00	10
18:00	30	10	10	39	26:35	10
15:15	30	10	10	39	17:07	10
13:30	30	10	10	39	21:15	10
8:00	30	10	10	39	17:20	10
00:15	30	10	10	39	32:30	10

To make my results more reliable I took more than one reading for each experiment. I wanted to find out how long it takes to each yeast solution of different age to produce a volume of 10 cm^3 of CO_2.
I collected the results at the same volume of carbon dioxide every time, so it will be easier to compare and analyse the results.
The volumes registered are: 0.50 cm^3, 2 cm^3, 5 cm^3 8 cm^3, 9 cm^3 and 10 cm^3.
I registered the time for each reading using the minute and the second when the particular volume was reached.
For each experiment I used the same scale to measure the amount of glucose, so there is no doubt about the quantity used – different scales have different sizes of error, so if there is any error on display, it will be constant and will not influence my experiment.

The results are shown in the following tables:

Age of yeast (h:min)	Starts bubbling (min:sec)	Time (min:sec)	Volume of CO_2 (cm^3)
20:30	30:10		
		32:05	0.50
		35:16	2.00
		43:00	5.00
		50:43	8.00
		52:24	9.00
		53:00	10.00

Age of yeast (h:min)	Starts bubbling (min:sec)	Time (min:sec)	Volume of CO_2 (cm^3)
18:00	16:04		
		17:13	0.50
		19:00	2.00
		21:12	5.00
		24:08	8.00
		25:21	9.00
		26:35	10.00

Age of yeast (h:min)	Starts bubbling (min:sec)	Time (min:sec)	Volume of CO_2 (cm^3)
15:15	05:50		
		06:27	0.50
		08:53	2.00
		13:30	5.00
		16:15	8.00
		16:35	9.00
		17:07	10.00

Age of yeast (h:min)	Starts bubbling (min:sec)	Time (min:sec)	Volume of CO_2 (cm^3)
13:30	08:00		
		11:25	0.50
		13:40	2.00
		18:07	5.00
		20:12	8.00
		20:42	9.00
		21:15	10.00

Age of yeast (h:min)	Starts bubbling (min:sec)	Time (min:sec)	Volume of CO_2 (cm^3)
08:00	05:30		
		07:29	0.50
		10:30	2.00
		13:15	5.00
		15:32	8.00
		16:43	9.00
		17:20	10.00

Age of yeast (h:min)	Starts bubbling (min:sec)	Time (min:sec)	Volume of CO_2 (cm^3)
00:15	00:00		
		02:45	0.50
		05:30	2.00
		21:10	5.00
		29:58	8.00
		31:56	9.00
		32:30	10.00

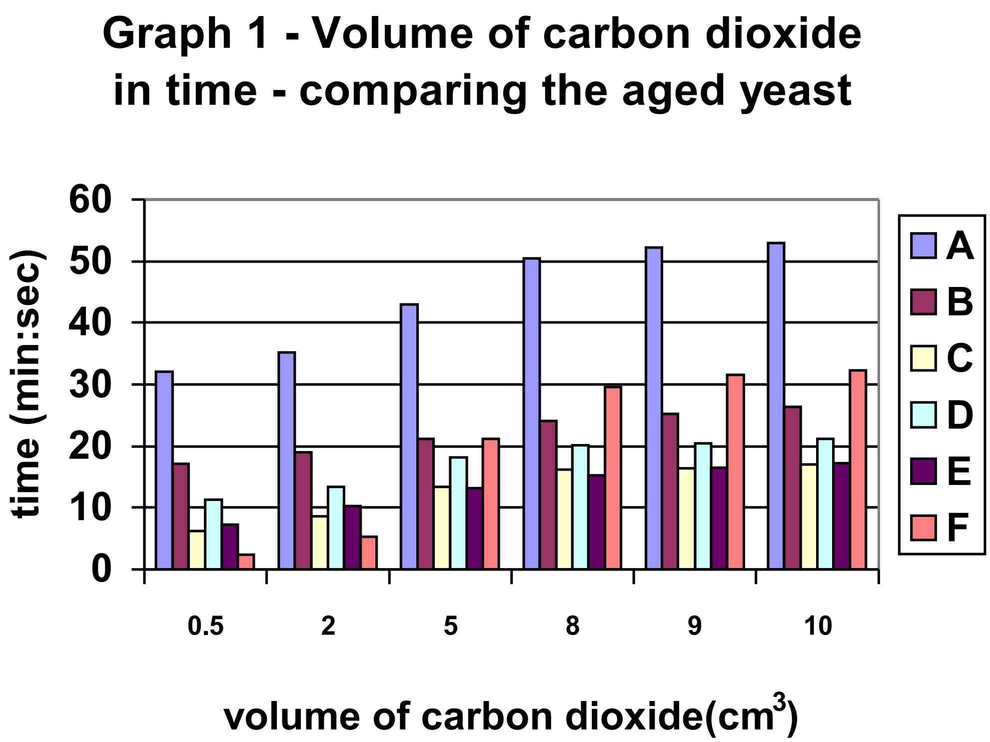
To work my results easier I'll write for each yeast a letter: A – the yeast is 20 hours 30 min old; B – the yeast is 18 hours old; C – the yeast is 15 hours 15 min old; D – the yeast is 13 hours 30 min old; E – the yeast is 8 hours old; F – the yeast is 15 min old.
To find out how much carbon dioxide was released in one minute I'll calculate the rate of carbon dioxide volume, per minute. The results are shown in the following table:

Yeast	Volume of CO_2 (cm^3)	Time (min:sec)	Rate of CO_2 volume per minute (cm^3/minute) to 1dp
A	10	53:00	0.188 ~ 0.2
B	10	26:35	0.376 ~ 0.4
C	10	17:07	0.584 ~ 0.6
D	10	21:15	0.470 ~ 0.5
E	10	17:20	0.576 ~ 0.6
F	10	32:30	0.307 ~ 0.3

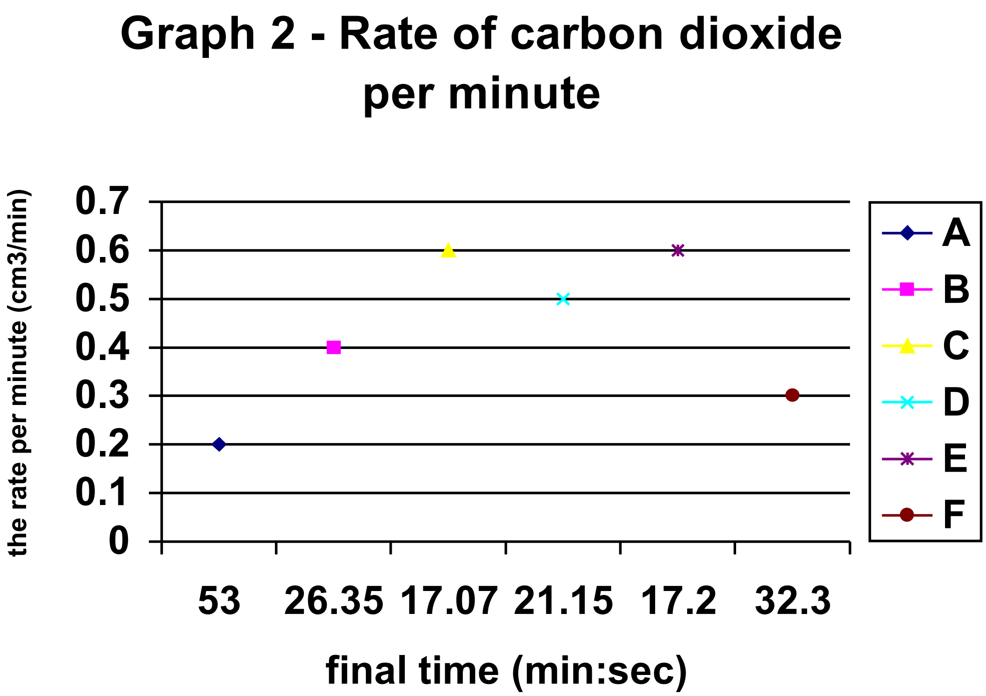
To find the rate of carbon dioxide per minute I divided the volume of carbon dioxide to the time. I transformed the minutes in seconds, and after I divided the volume to the time, I multiplied with 60, so the result will be in one minute. I recorded the rate of carbon dioxide per minute rounding to 1 decimal place, so it will be easier to represent the data in a graph.

Analysis

In my preliminary experiment I tried the apparatus and I concluded that it works out well, only that I had to make some changes during this experiment.
At the beginning my water bath was in a 100 ml conical flask, but I realized that the temperature was very hard to control (dropped rapidly) and I had to add warm water every two minutes, so I changed the conical flask with a larger one, of 250ml.
First I tried with a water bath 21˚C, but it didn't react, so I increased the temperature of the water bath to 39˚C and it started to react.



As we can see in this graph the eldest yeast (A) needs longer time to produce the same amount of carbon dioxide than the younger yeast, although as we can observe, the very young yeast (F) although starts reacting immediately, produces carbon dioxide in a slower pace, because to produce 5 cm^3 of carbon dioxide requires more time than the yeast right in front of it. In this graph we have two values that require more time D and F, while the other ones produce more carbon dioxide in shorter time. As I expected, the very fresh yeast needs more time to produce the same amount of carbon dioxide, because it had shorter time to respire anaerobically then the yeast that was prepared some hours earlier. I also have an odd value, that doesn't fit the trend, for yeast D, that was suppose to have a smaller value.
In the graph it is obvious that the very old yeast , A, takes a very long time to produce a certain volume of carbon dioxide; the second yeast that requires a long time is the youngest, F; the value for B follows the directly proportional increase of A, that also confirms the prediction.
The values for C, D and E separately follow the predicted trend, but put together it's not very clear how come C and E have almost the same values.



Graph 2 shows the rate of carbon dioxide produced in one minute.
The predicted graph would look like a parabola which does not touch the axes. If we draw the curve of best fit, the value for D do not fit the curve, so this is an anomalous data.
From graph 2 I can see that when time increase, the rate per minute decreases (as expected!), which also tells me that the older the yeast, the least carbon dioxide per minute is produced.

Fermentation is defined as an energy producing process by which organic molecules serve as both electron donors and electron accepters. The molecule being metabolized does not have all its potential energy extracted from it, which means that it is not completely oxidized
Most natural compounds are degraded by some type of microbe or can be attacked by bacteria. In environments lacking of oxygen (or other suitable inorganic electron accepter), this degradation involves fermentation.
Fermentation can involve any molecule that can undergo oxidation. Typical substrates include sugars (such as glucose) and amino acids. Typical products depend upon the substrate but can include organic acids (lactic acid, acetic acid), alcohols (ethanol, methanol, butanol), ketones (acetone) and gases (H_2 and CO_2).
When there is a shortage of oxygen not all the energy in glucose can be released and a waste product is formed.
Sugar = Carbon dioxide + Water + ethanol + Energy (in plant and yeast cells)

In the experiment the ingredients were mixed and then allowed to incubate at 24°C for a few hours. During this time the yeast should convert the sugar present to ethanol and CO_2.
In bakery, most incubations are for less than 4 hours not leaving enough time for the yeast to increase in number and at a temperature of 27°C. The CO_2 produced causes

the bread to rise (leaven) and become porous. The success of leavening is dependent upon the rate of gas production. This can be increased by adding more yeast, more sugar, or dough conditioners (various salts that the yeast need). Tweaking a recipe by manipulating these factors can speed CO_2 production, within reasonable limits. Adding too much of anything can either kill the yeast or cause the bread to rise too quickly. "The temperature of incubation is another critical consideration. *Saccharomyces* grows best at 26 to 28°C and deviations from that temperature will usually result in slow or complete lack of leavening. Failure as a baker can normally be attributed to either not adding the exact amounts of ingredients or inappropriate incubation temperatures during leavening."
All these apply to my experiment. The results are reliable, but I also have small deviations from my prediction, due to small errors in carrying out the experiment. I prepared the yeast solutions home, leave them over night and after that carry the solution to school, so during all this time I had no possibility to keep the temperature constant, so the yeast couldn't react properly all the time. I also carried out the experiment in two different days, and the last three results can also be influenced by this factor.

Evaluating

I think that the procedure used was very appropriate and reliable. I was able to collect the carbon dioxide in the measurement cylinder and record the values accurate.
My results confirm my prediction, with a single exception, the yeast solution labelled D, which do not fit the trend, in graph 2.
The procedure was appropriate, although small changes can be made to make the experiment more reliable. If I should repeat the experiment I would prepare the yeast solution in the laboratory a day before the experiment, put it by the heater, or in a warm place where the temperature is at least 26˚C – an appropriate temperature for the yeast to react; I will also record more than one results for the same age of the yeast and compare the results to see if they match and confirm the prediction.
Considering my results and analysis I can't draw a firm conclusion, because I should repeat the experiment again to record another value for the same age of the yeast. I also have to consider the anomalous result, repeat the experiment for that value and see if it was my mistake, or the result is the real value of that particular age of yeast. To provide additional evidence I should repeat the hole experiment in only one day for the same ages of yeast solution and repeat the experiment at least twice for each solution; put the results in a table, then in graphs and compare the results. Only after this I think that I can draw a realistic conclusion.

Reference:

Nelson Science Biology, by Michael Roberts & Neil Ingram

Introduction to Biology, Third Tropical Edition, by D.G. Macken

Biology at work, by Stephen Tomkins

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