Substrate on rate respiration in saccharomyces cerevisiae

The aim of this investigation is to examine what effects different substrates have on the respiration of yeast. I will investigate this by measuring the amount of carbon dioxide evolved during anaerobic respiration. The volume of CO2 gas will be collected using a gas syringe.

BACKGROUND INFORMATION

YEAST

Saccharomyces cerevisiae, also known as yeast, is a micro organism that uses saprophytic digestion to break down substrates. This is achieved through releasing specific enzymes to break down specific substrates, but if yeast does not contain a certain types of enzyme then it cannot break down its substrate. The more the enzyme of a particular substrate, the faster the rate of breakdown and therefore the more CO2 is produced. This will help me to test how much CO2 each substrate produces. Yeast can also respire aerobically and anerobically depending on the availability of O2. If there is plentiful of O2 then yeast would respire aerobically with sugars, producing H2O and CO2 as waste products. However, if no oxygen is available then the fermentation would occur which converts sugars into CO2 and ethanol.

RESPIRATION

Respiration is the process by which energy is released energy from glucose in the presence of Oxygen, forming carbon dioxide and water as waste products. Glucose releases energy in a series of reactions that take place inside components of the cell. The stages are briefly explained below:

GLYCOLYSIS

To get the sugar in a more reactive form it is produced to fructose-1, 6-bisphosphate by the addition 2 phosphate molecules. This process is a phosphorylation reaction. The fructose-1, 6-bisphosphate is then broken down into 2 molecules of glyceraldehydes-3-phosphate, which comprises of 3C each. The glyceraldehydes-3-phosphate converted into pyruvate via the oxidation process where each GAL3P molecule releases 2 hydrogen ions and 2 electrons. The electrons are then transferred to NAD to produce NADH (reduced NAD) and the energy is used to produce 4ATP from 4ADP and 4Pi. Finally there is a net yield of 2 molecules of ATP, and 2 molecules of pyruvate which is used in the link reaction and 2 molecules of reduced NAD which carries on to the link reaction.

LINK REACTION

In the link reaction the 2 molecules of pyruvate leave the cytoplasm of the cell and enter the mitochondrial matrix. This is an oxidation reaction where 2 NAD molecules oxidise 2 pyruvate molecules into 2 acid molecules. These 2 molecules of acetic acid then go on to combine with 2 coenzyme-A molecules to form Acetyl Co enzyme A. in the end of this stage 2 molecules of reduced NAD form, 2 molecules of CO_2 is lost and most importantly, Acetyl Co enzyme A is formed through the conversion of pyruvate. This is then used in the next stage of respiration.

KREBS CYCLE

At the start Acetyl Coenzyme A , combines with Citrate Synthase an enzyme as well and a 4 carbon molecule called oxaloacetate, forming Citrate. Then,

Citrate goes through the process of oxidative decarboxylation which forms a 5 carbon molecule called oxoglutarate. at this point NADH is produced and CO2 is removed. In the latter stages of the krebs cycle, the oxoglutarate is changed into a 4 carbon oxaloacetate molecule. NADH is made and 1 molecule ATP is also made. The volume of CO2 that is produced in the krebs cycle is important as this is the dependant variable.

ELECTRON TRANSPORT CHAIN

In this stage all of the NADH and FADH that has been produced in the previous stages is converted into ATP. This takes place in the cristae of the mitochondria. The NADH and FADH electrons move. When the electrons pass from one carrier to another, a series of reduction and oxidation reactions take place which releases energy in the process. This energy is used to pump H+ ions from the matrix into the intermembrane space, thus creating a gradient where the concentration of the H+ ions in the intermembranal space is higher than it s in the matrix. The inner membrane contains enzymes called ATP Synthase and The H+ ions diffuse through these enzymes causing energy to be released which is used to synthesise ATP through phosphorylation. The process is called because the final terminal electron acceptor is oxygen which picks up the electrons from the chain and the H+ ion from the matrix to form H20 as a waste product. This reaction is catalysed by the enzyme Cytochrome Oxidase

For every NADH which enters the chain and is oxidised by NADH dehydrogenase, 3 ATP are produced. For each FADH that enters the chain, 2 molecules of ATP are made.

ENZYMES

Enzymes are proteins that can effectively increase the rate of a reaction by lowering the required energy (activation energy) needed in order for the reaction to occur. Enzymes have a tertiary structure which decides the shape of the active site. The substrate must be specific to the active site because if they were not complementary to each other, then the substrate can no longer bind to the active site, thus the enzyme substrate complex does not form. The performance of enzymes can be affected in several ways some of which I have explained below.

TEMPERATURE

An increase in temperature will cause an increase in the rate of reaction because both the enzyme particles and substrate particles have gained kinetic energy. This will result in the particles to move faster, thus increasing collision frequency and the numbers of successful collisions as the particles have the required activation energy. If the temperature rises above the optimum temperature then the enzymes can become denatured. This happens because the enzyme molecule vibrates more causing the weak hydrogen bonds (holding the 3D structure of the enzyme together) to break. This eventually leads to the shape of the active site being altered. Consequently, the substrate will not be able to bind with the substrate as the shape of the active site is no longer complementary so the substrate enzyme complex can not form. This is important in my experiment because if the yeast (enzyme) was to become denatured then it would not be able to bind with the substrate (e. g. glucose) and the reaction would not be catalysed,

preventing any CO2 from being formed. I must ensure that temperature is kept constant throughout.

PH

Another factor which can affect enzymes is pH. Enzymes also have an optimum pH which is pH enzymes work best at. Changing the pH can change the tertiary structure due to the number of H+ ion in an acid or the OH- ions in an alkali. These ions disrupt the hydrogen and ionic bonds between -NH2 and -COOH. This will cause the tertiary structure to break down and changing the active site in the process. Once again, the substrate will no longer be able to bind with the active site, hence no substrate enzyme complex will form. I intend to use a buffer solution which will resist any changes in pH.

SUBSTRATE CONCENTRATION

Increasing substrate concentration increases enzyme activity as they are more molecules to occupy the active site, thus a faster reaction. If more enzyme substrate complex forms then more CO2 will be produced. However this is occurs only for a certain period until all the active sites are saturated with substrates. Therefore an increase in substrate concentration will not result in a increase in the rate of reaction.

PLANNING

THE DEPENDANT AND INDEPENDENT VARIABLE

The dependant variable will be the volume of C02 produced during respiration and the independent variable will be the substrates that I decide

to use in the experiment. These are Glucose, Fructose, Maltose, Lactose and Sucrose.

NULL HYPOTHESIS

The substrates will have no effect on the volume of CO2 produced during the respiration of yeast.

HYPOTHESIS

As the substrates are changed, the volume of CO2 formed during the respiration of yeast will also change

PREDICTION

I predict that of all my substrates, maltose will produce the greatest volume of CO2 when added to yeast in a fixed amount of time. Referring to my background knowledge, I know that glucose and fructose monosaccharides which can be directly absorbed by the yeast as no enzymes are required to break them down. This will allow for glycolysis to take place quicker. However I think that glucose will produce CO2 quicker than fructose because glucose is the main food source/ respiratory substrate for yeast, thus there will more glucose carrier proteins present in yeast. If more carriers are present then will enable absorption to occur quicker, hence respiration will happen quicker. So I believe glucose will produce more CO2 than fructose within a given time period.

However in terms of volume of C02, I believe maltose will exceed both of these monosaccharides. Maltose is a disaccharide that consists of two glucose molecules held together by a glycosidic bond. Once this bond is

broken down by maltase, there will be twice as many glucose molecules available in the same volume of other substrates such as glucose. More sugars can then be provided for respiration, hence more CO2 produced in 45 minutes. One point that must be taken into to account is that maltose can't be used directly, so it could take time before the glucose can be used. In addition, as glucose is a polar molecule it must be transported via facilitated diffusion. This could be a limiting factor if all the carriers become occupied, which would slow down the respiration process as a result.

After fructose, I predict sucrose will be the 4th substrate to produce the most CO2. Sucrose is also a disaccharide which consists of a glucose and fructose molecule. This substrate also requires enzymes to break it down and this could be a time consuming process as there is a limited amount of time. Furthermore, there aren't as many fructose carrier proteins present in yeast cell membrane compared to glucose.

Finally I predict lactose will produce the least amount of CO2 purely because yeast doesn't contain the enzyme lactase to digest lactose. This means that its monomers galactose and glucose cannot be used in respiration, thus no CO2 will be produced as a by-product.

APPARATUS

The following apparatus will be used when conducting the experiment:

Clamp and stand

Gas syringe – accurate to 0. 5cm^3/mol

Water bath – heated to 400C

Dry Yeast

Thermometer

Boiling tubes

Safety goggles

Universal indicator

Distilled water

Buffer solution (slightly acidic)

Substrates

Electronic weighing balance (2 d. p)

Stop watch

Rubber bung and rubber tube

Pipette (15cm3)

funnel

Measuring cylinders (250cm3)

Stirring rod

Conical Flask

Beakers (250cm3, 20cm3)

METHOD OF INVESTIGATION

Steps

Accuracy

Reason for method

1. Clean all the apparatus used to contain sugars or yeast using distilled water. Set up water bath at a temperature of 40°C.

N/A

Cleaning with distilled water ensures that all the equipment to be used in the experiment is clean and is free from impurities that could possibly interfere with CO2 collection. The water bath will be set to 400C because this is the temperature that I have decided to use in my experiments.

2. Fill up a 1 litre beaker precisely up to the 1litre mark with distilled water. Then add a buffer tablet into the beaker and stir thoroughly with a stirring rod

Make sure the distilled water has been filled up exactly to the 1L mark.

This is the step on how to produce a buffer solution. A buffer solution is required as it dissolves the yeast and substrate together. Allowing collision of the yeast and the substrate is vital otherwise a reaction would not occur

3. Weigh 30g of dry yeast using an electronic balance and transfer it into a beaker.

The scale will be accurate to 2 d. p. to allow consistency. If a solution contains more yeast, then more collisions may be involved between the enzymes and substrate, hence a greater rate of respiration, and more CO2 being produced than there should be.

30g of yeast will provide a stock solution for all 15 experiments, thus each experiment will use 2g of yeast. Excess yeast cells in the solution, will cause a large volume of CO2 production as more respiration will occur so 2g is a suitable amount. Keeping a constant concentration of yeast will ensure that my test is fair because an increase in yeast concentration will increase the amount of cells respiring therefore the volume of CO2 will increase

4. Place 250cm3 of buffer solution into a 300cm3 beaker containing dry yeast. Stir thoroughly

Ensure that the volume is read from the bottom of the meniscus level. The volume must be read at eye level

I have decided to use a bulk buffer solution because it keeps the concentration of yeast constant. Errors are more likely to occur if I had to weigh 2g of yeast and 15cm3 of buffer solution before each experiment. I have also taken into account of any spillages that may occur so I have ensured that I have prepared more than the required amount.

5. Accurately weigh the amount of substrate needed using the electronic balance and place into a 20cm3 beaker. Then, using a pipette, collect 15cm3 of buffer solution into a measuring cylinder and add it to the substrate

beaker. The solution should be stirred and the beaker should then be placed in the water bath.

Before using the balance confirm that it has been adjusted to 0. The measuring cylinder will be accurate to 0. 1cm3. Again, ensure that the reading is taken from the bottom of the meniscus and at eye level.

I must weigh the correct amount of substrate so that the concentration remains constant throughout the experiment (1M)

6. I will Prepare the conical flasks and attach the rubber tubing (connected to the rubber bung) to the gas syringe. I will carefully measure 15cm3 of yeast solution with the aid of a pipette and transfer it into a conical flask. This will then be stirred thoroughly and placed back into the water bath.

Pipette is accurate to 0. 5cm3.

The yeast has to be measured very accurately otherwise this would affect my results. For example if too much yeast is added, then there would be increase in amount of enzymes available and so there would be increase in successful collisions resulting in a faster rate of reaction with more CO2 being produced per unit time. The solution has to be agitated to ensure that the yeast molecules are evenly spread and do not settle to the bottom of the boiling tube – so that the chances of collisions increases.

The water bath will maintain the temperature of the yeast solution. This will prevent the enzymes from being affected by a change in temperature.

7. Use a thermometer to measure the temperature of both the water bath and yeast solution to ensure they are both 400C. As soon as the substrate is poured into the conical flask containing the yeast, immediately attach the bung onto the flask. This should be followed by timing using the stop watch.

The stop watch is accurate to 0. 01seconds. I have considered the difficulty faced when applying the rubber bung and starting the stop watch. I must make sure that I start the stop watch as soon as the bung is placed in postion and I intend to keep this the same for my other experiments.

A rubber bung and the rubber tube will be attached instantly as respiration can occur immediately. The CO2 produced will be collected in the gas syringe. It is important that does not escape. if this did happen then a smaller volume of CO2 would be collected by the syringe, thus the results obtained would not be precise. The stop clock will need to be started immediately to ensure all the experiments go on for exactly the same amount of time, if one experiment was to go on for longer more CO2 would be produced and thus I would get anomalies in my results. To avoid this, the clock needs to be started as soon as the experiment begins.

8. Take readings after 5 minutes of the co2 collected into the gas syringe with the aid of a stop watch. This step should be repeated until the 45th minute for each experiment. The temperature of the solution must also be taken, which should remain constant at 400C.

The readings must be taken straight after each interval. For example, I would take the reading just before the 5th minute interval. The Stop watch is accurate to 0. 01seconds.

The purpose of this step is to observe how much CO2 each substrate produces as time passes.

9. Using a universal indicator I will measure the PH after testing each substrate. The PH should constant throughout but if not, then it should still be recorded. The beakers, conical flask and measuring cylinders should be rinsed with distilled water after each experiment

NA

It is important to control PH as it could affect the amount of CO2. I will talk about this in greater detail in controlled variables. Rinsing removes any residue that may have been left over in the equipment

CONTROLLED VARIABLES

Controlled Variable

How I will control it

Why I will control it

Temperature

This variable will be controlled using a water bath which will be set to 40°C throughout the experiment.

The temperature must be controlled because the temperature will affect the rate of respiration of the yeast. If the temperature is changed, for example, too high then this may denature the enzymes used by yeast to digest substrates.

pH

The purpose of a buffer solution is to resist any changes in pH, therefore I will control the pH by add the yeast and substrate to a buffer solution.

When CO2 is released, it would dissociate, forming hydrogen ions and hydrogen carbonate. These will cause the pH to decrease and become more acidic. A decrease in pH would affect enzyme activity as this disrupts the charges (H+ and OH-) on the enzymes. This will result in changes in the ionic and hydrogen bonds holding the enzyme together. The enzyme would denature, thus the substrate will no longer fit and so an enzyme-substrate complex will not form.

Concentration of yeast used

I will prepare stock solution of yeast (30g) containing 250cm3 of buffer solution. This variable can be controlled by simply keeping the amount of yeast (15cm3) used constant throughout the experiments.

A stock solution will automatically eliminate any changes to the concentration of yeast since I will be taking the same amount of yeast from the same solution so it will always remain the same. Maintaining the concentration ensures that the same surface area is exposed by the yeast over which enzymes are released for extra cellular digestion to take place.

Concentration of the substrate

15cm3 of a 1M substrate solution will be used constantly.

If more substrate is added then more C02 would be produced. This is because there is more substrate available for the yeast to digest for

respiration, hence producing larger volumes of C02 than it should. If this variable is not controlled then it I would not be able to determine if an increase in CO2 is due to the type of substrate increase in concentration.

Timing

I will time the experiment using a stop watch in all of my experiments. I will constantly time the experiment for a total of 45 minutes, ensuring that the reading is taken immediately after each 5 minute interval.

I have to control this factor because if the yeast is left in the substrate for a longer time period for one experiment then this will allow more respiration to occur. The yeast will digest the substrate to produce more CO2 so therefore all solutions must be left to respire for exactly the same amount of time in order to obtain reliable results.

Culture of yeast

Use the same brand of yeast in all experiments.

Different types of yeast may cause different results as the number of carrier proteins may vary for a particular substrate. Using the same yeast will ensure that the size of the yeast in each experiment remains the same.

CALCULATING SUBSTRATE CONCENTRATION

In order to keep the substrate concentration the same I will have to calculate the mass of each of my substrates. Firstly, I will use the following equation:

Moles = Molarity x Volume

1000

The substrate concentration I will be using will be 0. 5M and the volume will be 25cm3. In order to determine the mass from the number of moles I shall then use:

Mass = Moles x Mr

CALCULATIONS FOR GLUCOSE AND FRUCTOSE

1000 0. 5 X 25 = 0. 0125mol

Fructose and Glucose has the same Mr of 180

0. 0125 X 180 = 2. 25g

I need add 2. 25 of each substrate into 25cm3 of buffer solution. I will produce a stock solution which will help maintain the concentration of the substrates throughout. I will be carrying out 2 experiments for Glucose or Fructose so I will need 4. 5g of each (2 X 2. 25 = 4. 5g).

CALCULATION FOR MALTOSE, SUCROSE AND LACTOSE

10000. 5 X 25 = 0. 0125mol

Maltose, Sucrose and Lactose have the same Mr of 342

Maltose, Sucrose and Lactose are isomers consisting of two monosaccharides linked together by a glycosidic bond. I have taken into account that when a condensation recaction occurs to form this disaccharide then a water

molecule is removed so I must substract the Mr of a water molecule from the Mr of the disaccharide. 360-18= 342

0. 0125 X 342 = 4. 28g

So I will add 4. 28g of Maltose, Sucrose and Lactose with 25cm3 of buffer solution. I will also produce a stock solution which will allow me to carry out the required amount of experiments. Therefore, I need to measure 8. 56g (2 X 4. 28g = 8. 56g) of each substrate which will then be dissolved into buffer solution.

CONTROLLED EXPERIMENTS

I have decided to carry out 6 controlled experiments for each of the 5 substrate in conjunction with the normal experiments. I will conduct these experiments in order to demonstrate and prove that the process of respiration cannot occur without the presence of the respiratory substrate as well as the yeast. The first experiment will involving a boiling tube containing only the 25cm3 of yeast solution. After placing the boiling tube in the water bath (400C), I will then record how much CO2 is produced. This would be conducted in the same way as my method where I would take readings after every 5 minutes until the 45th minute has been reached. The other 5 control experiments will only consist of the substrates. I will measure 15cm3 of each substrate into separate boiling tubes. These will also be placed in a water bath and the volume of CO2 produced will be recorded at every 5 minute intervals for 45 minutes. No CO2 being produced will confirm that the yeast cannot produce CO2 with the presence of a substrate and a substrate cannot respire on its own.

DATA ANALYSIS

Below is an exemplar table which will be used to analyse the results

produced in the experiment

This table will help me to calculate the average of the CO2 produced in each of the experiments after every 5 minutes. I will produce line graphs using the averages of CO2, which will enable me to compare the averages of the different substrates. From this, I can determine if digestion for polysaccharides and disaccharides effects how much CO2 is produced. This is how I will present the average of CO2 production for each substrate:

Graph showing the volume of CO2 produced against the time taken

Average CO2 Production (cm3)

Time (mins)

A t- test is a statistical test that takes a look the amount of data, if there is a difference between the means of two sets of data and also the spread of the data. A t-test is relevant as I will be using a large sample of results which will consist of results from other members in m class and including mine. The formula for the t-test is:

I have decided to construct a histogram for each substrate as this will allow me to compare my data easily after plotting frequency against experiments. A histogram will show if there any significant overlap between two substrates. Consequently, this can help me to make a decision of whether or not a t-test must be carried out. The below histogram would require a t-test:

Glucose

Maltose

MODIFICATIONS

I will use an inverted burette for measuring the volume of CO2 produced instead of a gas syringe. When carrying out my preliminary experiments I found that the gas syringe didn’t move smoothly, therefore i was unable to accurately read how much CO2 was being produced. Therefore I will use an inverted burette which has an inaccuracy of 0. 1cm3

Unfortunately, no buffer solution is available to control the pH of solution. In order to confirm that the pH hasn’t changed, I will measure the pH at the start and end of each experiment. This will achieved using a universal indicator.

I have changed the point at which I will take the reading. Initially I chose to take a reading jus before swirling the flask but I have now realised that this is incorrect. It would be wrong to do it this way because I want all the CO2 to escape from the flask before each reading is taken. So I will now take reading after swirling the conical flask.