The process of fermentation

Background research

Fermentation is a process carried out by many microorganisms and which produces a variety of useful compounds and this reaction is very important in industry for baking and brewing.

In fermentation, carbon dioxide gas bubbles out of the solution into the air leaving amixtureof ethanol and water. Ethanol can be separated from the mixture byfractional distillation. Fermentation must be carried out in the absence of air to make alcohol.

If air is present, ethanoic acidis made instead of alcohol. This reaction is very important in industry for baking and brewing.

Yeast, is most commonly used inbakingto break glucose, or other sugars to produce different products. In baking and brewing different type of yeast is used. An enzyme called invertase will convert a sugar called sucrose into smaller sugar molecules calledglucoseand fructose. Glucose is fermented by the yeast to ethanol and carbon dioxide.

The released carbon dioxide causes dough to rise and to hold it high. The produced alcohol contributes to the bread’s flavour. The optimal temperature for yeast to ferment sugar is 32°C. In warmer temperature (45 °C) the yeast cells will die.

Also fructose and sucrose care used by the yeast as fermentation substrates. Sucrose is directly transformed by an enzyme called invertase, into glucose and fructose. Sucrose is a good substrate for fermentation. When sucrose or glucose is added to the dough, they are faster fermented than maltose.

Sugars are small molecules which belong to the class of carbohydrates. As the name implies, a carbohydrate is a molecule whose molecular formula can be expressed in terms of just carbon and water. For example, glucose has the formula C6(H2O)6 and sucrose has the formula C6(H2O)11. More complex carbohydrates such as starch and cellulose are polymers of glucose.

The difference between a monosaccharide and a disaccharide can be seen in the following example:

How do enzymes work?

Enzymes speeds up the biochemical reactions and they work best at an optimum temperature, however if the temperature has increased it will provide more kinetic energy to the molecules involved. Therefore the number of collisions between enzyme and substrate will increase as well as the rate of reaction.

If temperature rises above the optimum the enzymes will be denatured. The bonds which are holding the structure together will break and the active sites lose their shape and will no longer react.

Reference

http://www. chemie. uni-regensburg. de/Organische_Chemie/Didaktik/Keusch/D-fermentation_sugar-e. htm

http://www. lycos. com/info/fermentation–sugars. html? page= 2

Investigating the affects of sugar on the rate of fermentation

The aim

To investigate on how different types of sugars can affect the rate of fermentation. There are two different types of sugars that I am going to which are monosaccharide and disaccharide sugars.

Introduction

Respiration is the release of energy from glucose or another organic chemical. The chemical energy in glucose can be used to provide the energy required for growth, repair and movement. This is a controlled process that occurs in small steps and each step requires respiratory enzymes. These enzymes allow the process to take place at body temperature 37C°. m

Aerobic Respiration is the normal form of respiration. It requires oxygen and releases the most energy from glucose. This form of respiration occurs within the mitochondria.

Glucose + Oxygen = Carbon Dioxide + Water + Energy

$C_6H_{12}O_6 + O_2 = CO_2 + H_2O$ + Energy

However, it is possible for respiration to take place without oxygen in a process known as anaerobic respiration. It also releases energy from glucose but not as much. When yeast respires anaerobically it produces carbon dioxide and alcohol. When we respire we produce lactic acid. Too much lactic acid causes fatigue to our muscles.

Yeast produces ethanol (alcohol) when it respires anaerobically and ultimately the ethanol will kill the yeast. We can respire in both ways too.

Normally we use oxygen, but when we are exercising, we may not get enough oxygen into our blood, so our muscles start to respire anaerobically. Word equation for anaerobic respiration:

Glucose lactic acid + Energy

$C_6H_{12}O_6$ $2C_3H_6O_3$ + Energy

Sugars can be categorized as either simple or complex depending on their chemical structure, in other words the number of saccharides (glucids) they are composed of such as:

Monosaccharide

Are the most basic unit ofcarbohydratesand they are the simplest form ofsugar.

Examples of monosaccharide includeglucose, fructose, and galactose. Monosaccharides are the building blocks ofdisaccharidessuch assucroseandpolysaccharides(such ascelluloseandstarch).

Disaccharide

Two monosaccharide joined together by a glycosidic linkage is called a double sugar or disaccharide. The most common disaccharide is sucrose. It is composed of glucose and fructose. Sucrose is commonly used by plants to transport sugar from one part of the plant to another.

Polysaccharide

Polysaccharides arepolymericcarbohydratestructures, formed of repeating units joined together byglycosidic bonds. These structures are often linear, but may contain various degrees of branching.

When all the monosaccharide in a polysaccharide is the same type the polysaccharide is called a homo polysaccharide, but when more than one type of monosaccharide is present they are called hetero polysaccharides. http://www. polypeptide-polysaccharide. com/

Hypothesis

I hypothesise that glucose sugar which is a monosaccharide will have a greater rate of fermentation than sucrose which a disaccharide sugar.

Justification

There are different types of sugars that have different effects on the replication of yeast, which would have an effect on the rate of fermentation. Therefore, I am going to investigate the main two sugars (Monosaccharide and disaccharides) to check which type of sugar will have a greater rate of fermentation. Monosaccharides are simple sugars made of 1 molecule of sugar whereas disaccharides are complex sugars made of two molecule of sugar.

So, my hypothesis would be that glucose will increase the rate of fermentation than sucrose because glucose is a monosaccharide sugar and therefore has one unit of sugar. This will enable the enzymes in the yeast to break down the bonds of the simple sugar very easily with less energy, and short period of time.

Whereas sucrose has two unit of sugars and therefore has twice as much bonds as glucose sugar which will slow down the enzymes' action in breaking down the bonds, as it requires more energy with longer period of time to break down the bonds.

So, in order to check whether my hypothesis is right or wrong, I will need to perform the experiment by testing the main two sugars glucose (Monosaccharide) and sucrose (disaccharides).

Experimental method

In the experimental method I have decided to use the technique of titration.

A titration is a technique where a solution of known concentration is used to determine the concentration of an unknown solution. So in this experiment, I am going to use the titration technique to find out which type of sugar will produce a greater rate of fermentation.

Typically, the titrant is added from aburetteto a known quantity of the analyte (the unknown solution) until the reaction is complete. Knowing the volume of titrant added allows the determination of the concentration of the unknown. Often, an indicator is used to usually signal the end of the reaction, the endpoint.

http://www. science. uwaterloo. ca/~cchieh/cact/c123/titratn. html

Here are some important apparatus that are important to carry out the titration method:

* Burette: The burettes are mainly used for titrations to deliver one reactant until the precise end point of the reaction is reached. Burette used to measure the volume of a solution accurately which can be read to an accuracy of half a division that is to 0. 05 cm3.

· Conical flask, beaker: The conical flasks, beakers are used for mixing, reactant and transporting but not for accurate measurements. The volume stamped on the sides of the conical flask and beaker is approximate and accurate to within 5%.

* Pipette: Pipettes are used to measure small amounts of solution very accurately and it has a bulb to draw the solution into the pipette. It transfers 25 cm3 (usually to ±0. 05 cm3) of a solution into a conical flask.

* Funnel: is a pipe with a wide, often conical mouth and a narrow stem (this will be needed to make sure the transferring of the sodium hydroxide into the burette in smooth and safe as possible).

* 0. 1M of sodium hydroxide: will be used as the solution in the burette which will indicate the amount of alkali that is needed to neutralize the acid in the fermented solution.

* Phenolphthalein indicating solution: this indicator solution will help us see when the solution in the conical flask changes, it is very important that we use the same amount of drops on both solutions this will help us get an accurate colour change result.

Apparatus:

* 2 g dried brewer’s yeast.

* 200cm 0. 2 M fructose.

* 200cm 0. 2 M lactose.

* 2 x 0. 5 g ammonium phosphate.

* 2 x 0. 5 g ammonium sulphate.

* 3 x 250cm wide necked conical flask.

* 2 x silicone rubber bung with two holes.

* 3 x glass fermentation lock.

* 3 x 15cm bent glass pipette with 3cm rubber tubing.

* 3 x restriction clip (Hoffman clip).

* 3 x glass rod.

* 50cm burette.

* 3 x pipettes.

* 0. 1 M sodium hydroxide solution (about 400cm).

* Phenolphthalein indicator solution and dropping pipette.

Procedure for day 1:

1. Label two 250cm flask: fructose and lactose and control (water). Add 200cm of 0. 2 M sugar solution to the named flasks and 200cm of water to the control flask.

2. Add 2 g of dried brewer's yeast and then 1 g of ammonium salts to each flask (0. 5 g each of ammonium phosphate and ammonium sulphate).

3. Ensure that the yeast is respuspended and the salts are dissolved in the sugar solution by carefully stirring each solution with a different glass rod.

4. Carefully and firmly insert the fermentation lock and bent pipette into the silicone rubber bungs.

5. place the bungs firmly into the neck of the flasks

1. To assist the fermentation the flask should be placed in an incubator (15 – 20 C).

Procedure for day 2:

1. Set up a burette containing 0. 1 M sodium hydroxide solution.

2. Swirl the flask to ensure a homogenous mix of culture and remove a total of 25cm of sample (10cm + 15cm).

3. Place the removal sample into a small flask and add two or three drops of phenolphthalein solution.

4. Plot a histogram of the volume of the alkali used to neutralize each sugar solution. The histogram can be used to indicate the extent of fermentation.

Justifying day one procedure:

There are few things that can affect the preparation of the solutions which are usually known as a potential errors and these error can come from:

Weighing balance: we used the 2 decimal place balance to weigh our samples and I think the weighing of the sample would not be reliable as it measures to 2 decimal places. In this case our results might be unreliable

because we cannot determine whether it is the exact weight of the sample we are measuring. For example if we weighed out 3g of yeast on the 2 decimal place balance it would only show 3. 00g, whereas if we used another balancer which measures the sample to an accuracy of 4 decimal places it would have been better because it would give us 3. 0000g.

Stirring rod: depending on the pace of stirring the solution if we didn't use the stirring rod gently and frequently it would affect the solubility of the sample that we are making, this is because sometimes we may think that all the solid part in a solution are fully dissolved in the sample. However, sometimes a small amount of the solid may not dissolve properly without noticing it. Therefore, it is very important that we had to stir the solution gently and frequently so that the entire solid are completely dissolved.

Room temperature: leaving the solution to ferment over night the temperature of the room is not constant because at night the temperature decreases which would have an effect on the rate of reaction of the fermentation by slowing the reaction down. It would have been better if I could use a water bath so we can take a full control of the temperature and also make it constant.

Duration for fermentation: the duration that was provided for fermentation was not enough for the yeast to ferment, if the solution was left for longer period time the sample might have fermented better and also if would have left the solution for longer night probably 2 to 3 nights it would have been better too. However, leaving the samples for more than 4 to 6 nights could

affect the rate of fermentation because the longer we leave a sample the more contaminated the sample may get by bacteria.

Justifying the procedure of day 2:

In day 2 we have used the technique of titration to find out which type of sugar will produce a greater rate of fermentation. However, the manual titration technique is not as accurate as it is industries. The titration technique is carried more accurately on an industrial scales because of the automated machines that are used are automated which carry out the titration in a more accurate way and more than one sample at a time.

The titration method: the method only allows us to do one titration at once which was not suitable for our time scale. We were using two burettes one for each solution but we still had to run one burette at a time.

Time: I think the period of the titration was not sufficient because we had to carry out

three titrations and three repeats for each type of sugar including the control, keeping in mind that we had to record the all values accurately from the titration. Therefore, we would rush in the experiment to finish all the titrations as quickly as we possibly can, so we would not carry out the investigation in an appropriate way which could affect our overall result.

Recording the results and how many repeats will be performed

In this investigation I will be using two types of sugars which are glucose and sucrose and a control which is water. For each type of sugar including the control I will make 3 repeats so that I can get an average result of the volume of the sodium hydroxide which has been used. I would perform a

rough titration for each sugar to help me to decide approximately where the end point is going to be and how much volume of the sodium hydroxide will I need to neutralise the solution that I am testing

Type	**Titre1 Cm³**	**Titre2 Cm³**	**Titre3 Cm³**	**Average Cm³**
Glucose	22. 65	34. 85	25. 90	27. 80
Sucrose	52. 00	40. 45	40. 750	46. 73
Control	8. 15	17. 60	8. 15	11. 30

Once I have completed the experiment and recorded my results accurately to two decimal places, then I will work the average result for both sugars and the control for example, for glucose sugar I would add the results that I have obtained including the rough one and then divide the answer by three. Once I have calculated the average result for both sugars and the control, then I would plot a graph to show the volume of sodium hydroxide that has been used to neutralise each solution which will help to compare which type of sugar fermented better. Titration results

Conclusion from the results

During the titration process I kept watching for the colour of the solution we were titrating to change from cloudy white solution to a light pink colour. The light pink colour indicate that that neutralisation of the solution we are tittering is completed which known as the end point.

Looking at this table it shows that sucrose has a greater rate of fermentation than glucose because it has a higher titre of sodium hydroxide that was needed to neutralise the solution. Therefore, this indicates that sucrose was more acidic and more CO_2 dissolved in the sample that we were testing and also more fermentation rating took place.

Accuracy of procedure and each piece of equipment used

Each piece of equipment we have used, we take the volumes reading from the bottom of the meniscus. Burette used to measure the volume of a solution accurately which can be read to an accuracy of half a division that is to 0. 05 cm^3.

* Rinse equipments before use: We have used distilled water to rinse the equipment before we carry out our investigation because the equipment may not washed properly so it contains other solutions which would make our results unreliable. By rinsing the equipment before using them, would decrease the possibility of getting of contamination.

* Labelling equipments: We had to label the conical flasks to ensure that the right sugar is in its labelled conical flask because sugars look the same so labelling conical flasks would help us identify the solution quickly without getting mixed up of which sugar belongs to which flask .

* Ammonium salt: As we know that yeast gets food from the surroundings and therefore, we have used the ammonium salt and ammonium phosphate is to feed the yeast with nutrient as ammonia contributes to nutritional needs of such organism.

· Using room temperature for fermentation: Because enzymes within yeast are from different habitats therefore using different temperatures for each type of sugar would affect the fermentation process. Therefore we decided to use room temperature as it is suitable for both types of sugar and the yeast in which perform the fermentation process.

· Swirling flasks: It is very important that we had to swirl the flasks properly before taking the samples out because it would help ensure that all the solids are fully dissolved in the solution and becomes complete solution.

· Using pipette filler to take the samples: we would be using pipette filler because it is good equipment for taking around 25cm3 of the solution.

· Phenolphthalein indicator: We have used this indicator solution to help us to see when the solution in the conical flask changes, so we had to use the same amount of drops on both solutions so that we get an accurate colour change result.

Evaluation:

The reliability and the accuracy of the investigation:

It is very important that we had to follow all the instructions carefully that were provided to us because it would help us work more accurately and get better result on our experiment. However, we would not expect to get the same results for each repeat of titration, because it depends on determining the end point of the reaction.

For example, the cloudy white colour is quite similar to the light pink colour therefore; sometimes it is difficult to determine whether the exact end point

has been achieved or not , and so we wouldn't expect to get the same results for each time we repeat the experiment. As a result, it would be better to hold the solution up to the light to help us determine the exact end which is the light pink colur in the same range.

As we know that yeasts perform better under anaerobic conditions, so if oxygen got into the solution then the condition inside the conical flask will change to aerobic and the process of fermentation will not take place.

As a result, we had to ensure that the process is taking place with the absence of oxygen conditions, so we ensured that the bung was firmly fastened into the conical flask that contained the fermenting solution. It was very important that that the bung was fastened otherwise the air that came from the surrounding would affect the yeast respiration by getting into the conical flask to the solution that we were fermenting.

Moreover, if the bung is not fastened properly then carbon dioxide will leak from the conical flask would affect on the acidity of the solution because the sodium hydroxide needs to be titrated with an acidic substance so to achieve neutralisation of the solution in the flask. Therefore, keeping the bung fastened will keep the process of fermentation under anaerobic condition.

When the samples had been left to ferment overnight, bubbles were produced on the top of the solution because the bubbles were formed from the carbon dioxide gas being given off from the reaction in the solution. This may have an effect on the measurement of the solution in both the pipettes and burettes because the solution must be measured from its meniscus. Therefore we have got to be careful while taking the reading of the solution

to take from the meniscus which is the curve at the top of the liquid if did so we would get more accurate and reliable results.

There is another factor which can make our investigation unreliable which the temperature. This can have a major effect on the rate of fermentation because enzymes are very sensitive to temperature. Enzymes speeds up the biochemical reactions and they work best at an optimum temperature, however if the temperature has increased it will provide more kinetic energy to the molecules involved. Therefore the number of collisions between enzyme and substrate will increase as well as the rate of reaction.

If temperature rises above the optimum the enzymes will be denatured. The bonds which are holding the structure together will break and the active sites lose their shape and will no longer react.

There are some factors in which can have an effect on our overall result such as, room temperature, weighing and the concentration of the samples. So Now I going to make a table to show the variables, the effects they may affect the investigation and how they can be controlled during the experiment to get more accurate and reliable data.

Controls and variables during this experiment:

Variables	The effects on the experiment	How could it be controlled
Room temperature	As we know the room temperature is not constant therefore it would affect the enzymes action during the process of fermentation	We could have made the temperature constant if we placed the samples inside an incubator which will help the enzymes work better.
Weighing	Another factor that could affect our overall result is that being very close	In order to optimise the effects of the air on the weighing balancer while we

to the weighing balancer while we are weighing our samples because breathing on the balancer changes the reading of the sample	are taking the reading of the sample is to use an accurate weighing balancer which is surrounded by glass frame and gives the reading of the sample to four decimal places.

Concentration of sample	If we used the wrong concentration of the sugars, this would affect on our results.	In order to make sure that we are using the right concentration we have look carefully at the labels of the solutions which indicates the name of the solution and its concentration.

Sources Used

http://www. chemie. uni-regensburg. de/Organische_Chemie/Didaktik/Keusch/D-fermentation_sugar-e. htm

http://www. practicalchemistry. org/experiments/fermentation-of-glucose-using-yeast, 109, EX. html

http://www. chemie. uni-regensburg.

de/Organische_Chemie/Didaktik/Keusch/D-fermentation_sugar-e. htm

http://www. daviddarling. info/encyclopedia/P/polysaccharide.

htmlhttp://www. gcsescience. com/rc17-fermentation-yeast-alcohol. htm