

Yeast and different carbohydrate substrates essay sample

[Science](#), [Chemistry](#)



**ASSIGN
BUSTER**

Hypothesis.

The hypothesis that I draw is that "" out the five carbohydrate substrates that I will use, Glucose will produce the highest volume of Carbon Dioxide at every five-minute interval.

Null Hypothesis.

The null hypothesis that I am composing is that "" the five carbohydrate substrates that I am to use will not produce any Carbon Dioxide.

Scientific Research.

Under anaerobic conditions (when Oxygen is unavailable) yeast cells will break down carbohydrate substrates into ethanol and Carbon Dioxide. This is known as fermentation and is explained using the following equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{Energy (210kJ)}$$
 The process consists of a series of reactions catalysed by specific enzymes "" that are chemicals produced by yeast cells. The enzymes produced by the yeast will only catalyse the fermentation of certain carbohydrates but not others. Moreover, different species of yeast produce different enzymes.

Enzymes work using the enzyme-substrate complex. That is, the substrate becomes attached to the enzyme using the active site of the enzyme using the lock and key theory. This allows the substrate and the enzyme to interact in a specific way leading to a chemical reaction involving the substrate to take place and appropriate products will be formed.

Enzymes are highly specific and so they have the ability to distinguish between pairs of optical isomers (and this is important in this case). A close match between the structure of the substrates and the outline of the active site helps achieve this. However, enzymes are proteins and since proteins are not rigid, some enzymes are able to fold around their substrate after the initial binding.

The two most important substances that one must consider (in relation with this study) are zymase and co-zymase, that are both found in yeast. These enzymes will be used by the carbohydrate substrate to form products such as ethanol and Carbon Dioxide. Along with zymase, co-zymase has to present and this is required to catalyse the reaction successfully.

Arthur Harden and William Young "" according to Lubert Stryer (1975) made the discovery of zymase and its relation with fermentation in 1905. Harden and Young found that on adding yeast juice to a solution of Glucose, fermentation started immediately. This has led to the theory that "" zymase catalyses the change of glucose in to ethanol and Carbon Dioxide.

Before 1905, Hans Buchner and Eduard Buchner had carried out many key experiments related to fermentation. According to Stryer (1975) "" these two scientists made a discovery of fermentation (by accident) but they had been using sucrose. The disadvantage with this is that it is much slower than in producing products than glucose.

Below: During fermentation, the carbohydrate substrate is broken into ethanol and Carbon Dioxide using electron carriers such as NAD+.

Glucose appears to be a favourite of many scientists who have dealt with fermentation. For example, the wine industry use glucose as a substrate for zymase to interact, in order to produce the relevant ethanol and Carbon Dioxide.

Below: Often to obtain Glucose in industry a series of reactions are carried out using starch.

The above process is capable of producing other six-carbon sugars such as Fructose "" however, commercially glucose is favoured.

Overall, one cannot state that only Glucose is able to convert into ethanol and Carbon Dioxide, as this is not true. Other Carbohydrate substrates such as Fructose, Galactose and so on are also capable of this reaction. However, with strong evidence that those who have dealt and deal with fermentation prefer/red Glucose as their carbohydrate substrate "" one assumes that it is the most efficient and that the structure of Glucose is one that is required for yeast enzymes to work. This is what this study is aiming to prove that Glucose has the most favourable structure for yeast enzymes to work. Using evidence from the past and scientific background the hypothesis that has been developed for this study "" favours Glucose.

Key Factors Affecting the Experiment.

In order to compose a ""fair and true' study one must consider the key factors that will have an affect on the final results. Moreover, one must decide which factors to control (i. e. keep the same throughout the

experiment) and which factors to use as variables (i. e. factors that are subject to change).

Temperature: This is a key factor for this experiment, as variations in temperature will directly affect the reaction rate. If one increases the temperature, they will increase the reaction rate. This is due to the increase in the kinetic energy of the reacting molecules. Hence, more enzyme-substrate complexes are formed in the same amount and so products will be formed faster. However, since enzymes are involved one has to consider denaturation. That is, if the temperature is increased above the enzyme's optimum temperature (tends to be 37°C) then reaction rate will decrease. This is due to bonds (involved in enzymes) becoming unstable and so the contour of the active site becomes distorted and so the enzyme-substrate complex becomes harder to form. In this experiment, the temperature will be controlled, that is the experiment will take place under the temperature of 35°C.

pH: This other key factor has to be considered when dealing with enzymes. Most enzymes have an optimum pH and this is when the reaction rate is at its highest point. Little fluctuations in pH have a great effect on the reactions rate. That is, some pH values are inhibitory and cause the loss of interaction of the enzyme with the substrate. The other affect of pH changes is that some values of pH weaken bonds holding the enzyme and cause the enzyme molecule to change shape. Hence, the chance of interaction is lowered. In this experiment, the pH will be controlled and so all the carbohydrate will be working under the same pH and this will be pH 7.

Enzyme concentration: This factor refers to the amount of enzyme molecules involved in the reaction. This factor will have an affect provided a large substrate concentration is available because this will mean that more enzyme molecules are available for the substrate (to react with) and so an increased reaction rate will be resulted. However, if a low substrate concentration is present, it will mean that a limited amount of interactions can take place. That is, the reaction rate will increase to a certain point and will remain at this point (V_{max}). In this case, the yeast will provide the enzymes required and so the amount of yeast used "" will have to be controlled.

Substrate concentration: The biological principle of this factor is similar to the principle of enzyme concentration. That is, if there is an excess of the substrate and a large concentration of enzyme is available it will result in an increased reaction rate. However, if a low substrate concentration is present, it will mean that a limited amount of interactions can take place. That is, the reaction rate will increase to a certain point and will remain at this point (V_{max}). In this case, the concentration of all the carbohydrate substrates to be used will be controlled.

Types of yeast: There are many different types of yeast and these include: Baker's yeast, Dried yeast, brewer's yeast and so on. These all will have a different affect and one may be faster in reacting than another. That is, one type of yeast has different substances than another and this may contribute in increasing reaction rate. In this experiment, the type of yeast will controlled "" i. e. the same yeast will be used for all the tests.

Surface Area: This refers to the surface area of the solid reactants and in this case they are yeast and the carbohydrate substrate. If the surface area is increased, the reaction rate will too be increased because more surface of the reactant is available for the reaction to occur and so the reaction rate will be increased. In this case, both reactants will have the same surface area.

Type of carbohydrate substrate: The type of carbohydrate substrate is extremely important in studies related to fermentation. That is, different substrates will produce different reaction rate. For example, disaccharides will be much slower in forming the products than monosaccharides as disaccharides will have to convert to monosaccharides first and then react with the enzyme. However, the number of units of carbohydrates is not the only factor. That is, two different monosaccharides (isomers) will have different reaction rate. This is due the difference in the molecular and structural formulae "" which will have great influence on the interaction between the substrate and enzyme. The structure of the substrate must compliment the shape of the active site to produce optimum results "" i. e. high reaction rate.

This will be the study's variable and so the unit of carbohydrate will remain the same but the type and the structure of carbohydrate substrate used will vary. That is "" Glucose, Galactose, Fructose, Sorbose and Mannose are all monosaccharides ($C_6H_{12}O_6$) but they all have different structures and so are isomers of each other.

Apparatus.

3 beakers 4 large test tubes Bunsen Burner Heat Proof Mat Tongs Test tube rack 10g Glucose 10g Galactose 10g Fructose 10g Sorbose 10g Mannose 6g dried yeast 20cm³ Phosphate Buffer Solution Janus Green B Delivery tubes Clamp Stand Boss Mortar and pestle Spatula Glass Syringe Anti-bumping granules Large conical flask Water bath at 35°C Rubber Bungs Rubber tubing 5 " 10cm³ Paraffin Stop-watch Stirring rod Pipette filler Measuring cylinder Electronic balance Plan Method.

After collecting the apparatus required " 200 cm³ of distilled water will be added to a beaker, along with 10 grams of Glucose " that has been weighed using an electronic balance (to make a 5% Glucose solution). After the Glucose has dissolved, the solution will be boiled until it reaches the volume of 150 cm³.

NB: This will be achieved by using the numeric marks on the beaker.

The 150 cm³ solution will be placed into the conical flask and it will be corked. The conical flask (containing the Glucose solution) will be placed into a trough that has cold water " to cool the solution. When the solution reaches the temperature of 25°C, 6 grams of yeast will be added and this will be corked. This solution will be shaken continually until no more lumps are visible.

20 cm³ of this solution will be transferred to another conical flask, along with Phosphate Buffer Solution (pH 7) and four drops of Janus Green B or Diazine Green solution (indicating presence of or absence of Oxygen). This mixture

will be covered by using 5 cm³ of Paraffin that will form a thick layer allowing for anaerobic respiration to take place.

This conical will be corked and will be placed in a water bath of 35°C. As soon as the Janus Green B solution turns pink (indicating absence of Oxygen) the vertical arm on the cork will be used to attach delivery tubes to the “conical cork” and will be connected to a glass syringe. This syringe will be placed outside the water bath and will be held by the boss on a clamp stand.

NB: It will be important to check that the glass syringe is marked down to 0 mm.

When the delivery tube is attached to the glass syringe “” immediately the stopwatch will be started. After five minutes has past on the timer, the volume of Carbon Dioxide gas collected in the glass syringe will be recorded. The results will be collected at every five-minute interval over thirty minutes.

This method will be repeated but Glucose will be replaced by Galactose, then Fructose followed by Sorbose and finally Mannose.

After all five carbohydrates have been tested the above method will be repeated four times “” to ensure accuracy.

Diagram: Outline of Results Table.

Carbohydrate	Volume Of CO ₂ (ml) Collected After	Substrate	0 mins	5 mins	10 mins	15 mins	20 mins	25 mins	30 mins
Glucose	4.5	13.5	22.0	40.0	78.5	98.5	100+		
Galactose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fructose	0.0	0.0	12.0						

27. 5 36. 5 49. 5 78. 0 96. 5 Sorbose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 Mannose
 Carbohydrate Volume Of CO₂ (ml) Collected After”| Substrate 0 mins 5 mins
 10 mins 15 mins 20 mins 25 mins 30 mins Glucose 7. 0 7. 0 24. 0 36. 0 73. 5
 95. 0 100+ Galactose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 Fructose 0. 0 13. 5 29.
 0 46. 0 46. 5 47. 5 49. 0 Sorbose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 Fructose 0. 0
 16. 5 36. 0 49. 0 69. 5 79. 5 98. 5 Method.

Apparatus 3 beakers Bunsen Burner Heat Proof Mat Tongs 10g Glucose 10g
 Galactose 10g Fructose 10g Sorbose Spatula Measuring Cylinder 6g Dried
 Yeast 1 Phosphate Buffer- Solution (pH 7) tablet Janus Green B Delivery
 tubes 4 Rubber Bungs Clamp stand Boss Glass Syringe Large conical flask
 Water bath at 35oC 2. 5 cm³ Paraffin Stop watch Stirring rod 3 Pipettes
 Thermometer Electronic Balance Procedure.

Before starting the experiment, all necessary apparatus were collected. Into
 a 250 cm³ beaker, 200 cm³ of distilled water were added along with 10
 grams of Glucose. The Glucose solution was boiled and stirred regularly till it
 reached to the volume of 150 cm³ and this was achieved by using the
 numeric marks on the beaker. This procedure was quite necessary as it
 dissolved the Glucose but more importantly it removed a fair quantity of
 Oxygen and this helped maintain the anaerobic conditions required for the
 experiment.

After boiling the solution, it was transferred into a conical flask and this was
 corked and placed in a trough, which had been filled with cold tap water.
 This water would help cool the Glucose solution and this was necessary

because if the yeast solution were added at this point, the yeast enzymes would denature due to the high temperature of the Glucose solution. The conical flask was corked to help maintain anaerobic conditions. However, the cork was regularly taken, since the temperature had to be checked. Once the temperature of the Glucose solution reached 25°C, it was taken out of the water.

Meanwhile, into another 250 cm³ beaker, 50cm³ of distilled water were added to 6 grams of dried Baker's yeast, to make a 12% solution. Using a stirring rod, the yeast was dissolved and then the Phosphate Buffer Solution (pH 7) had to be prepared. This was made by placing a Phosphate Buffer Tablet into 100 cm³ of distilled water. This solution was essential because it was required to maintain the pH Glucose/Yeast at pH 7.

The Glucose, yeast and Buffer solution had to be prepared by the biologist and every time, the quantity of solution prepared came to an end, more quantities of the solutions were prepared using the same procedures.

After all the solutions required were ready, into a conical flask, 20 cm³ of Glucose solution were added along with 20 cm³ of the Yeast solution and 20 cm³ of the Phosphate Buffer solution. Furthermore, four drops of Janus Green B were added and this mixture was shaken vigorously for five seconds. This procedure had to be dealt with caution as the Janus Green B is hazardous. Nevertheless, this mixture was then covered by adding 2.5 cm³ of liquid Paraffin and this formed a fairly thick layer over the mixture. This thick layer maintained the anaerobic conditions of the experiment, since the layer

prevented any Oxygen entering the mixture. In addition, the Janus Green B is an indicator of Oxygen, that is, it is dark blue in the presence of Oxygen and in the absence of Oxygen, it is pink.

The conical flask with the solution after it was ready was placed into a water-bath, which was at 35°C. In the meantime, the clamp stand was set up and this was needed for the syringe (needed to collect the gas). The clamp stand was set up outside the water bath, with the syringe being held by the boss that was on the clamp stand. The syringe before measuring, was marked to zero and this ensured accurate results to be collected. In addition to this, the syringe had a delivery tube attached to it with a cork at the end of the tube. The concept being that the cork would be placed onto the mouth of the conical flask (when it was ready) and as the Carbon Dioxide is produced through anaerobic respiration, it rises up and goes into the hole in the cork and through the delivery tube and into the syringe.

The moment the solution turned pink, the cork was placed onto the conical flask and the stopwatch was started. The pink colour (as mentioned before) was a sign that Oxygen is absent and this was desired, as anaerobic conditions were required. After five minutes had past on the stopwatch, the mixture was swirled once and the volume of Carbon Dioxide gas collected in the glass syringe was recorded in millilitres.

Furthermore, the results were recorded at every five-minute interval over a time period of thirty minutes and before checking the volume of Carbon

Dioxide collected, the mixture was swirled once. It was necessary to record the results over thirty minutes because the results are “fair” and valid.

The method was repeated but Glucose was replaced by Galactose then Fructose followed by Sorbose. After every carbohydrate substrate had one set of results, the whole method was repeated to produce a second set of results. It was essential to have at least two sets of results, so that an average set of results can be calculated. This would ensure fairness and accuracy in the investigation.

Key Factors Affecting the Experiment.

The investigation had the aim of investigating the different types of carbohydrate substrates and so apart from this variable, every other variable had to be controlled, that is, they had to remain constant throughout the investigation.

Proceeding through the investigation, there were many areas where it was necessary to remember and apply the above.

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Temperature must be considered when investigating enzymes and the first time the problem of the temperature was encountered was when the substrate solution was to be cooled. It had to be cooled to a temperature that was not too high, otherwise the yeast enzymes would be denatured and a temperature that is too low is not very efficient for enzymes. Hence, the temperature was allowed to fall to 25°C. This temperature would have most likely fluctuated over time (due to the room temperature) but this was not a problem, due to the fact that the solution was to be placed in a water bath. The only factor that had to be considered was that the temperature could not be too high or too low.

The yeast used had to remain constant and this was essential to remember as different types of yeast have different substances and so this factor could have interfered with the results. That is, wine yeast has different substances that may act differently with the substrate.

Moreover, the concentration of the yeast solution had to remain constant because varying the concentration of the solution could have distorted the results. That is, the yeast provided the enzymes required for the reactions to have taken place and so, if the yeast concentration had been higher (provided that the substrate concentration is high) the reaction rate would have increased and more Carbon Dioxide would have been produced

in a shorter time. This would cause great problems as the conclusion drawn from such results, would be inaccurate.

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However, every time more quantities of each solution were required, more was made but it was fundamental that the method used to make the solution was exactly the same throughout the investigation. Otherwise, the results may not be accurate and moreover, they may not be “‘fair and true’ results.

The volume of every solution that was used had to remain constant because varying the volume would have made the results inaccurate and “‘unfair.’ Therefore, throughout the investigation, 20 cm³ of substrate solution were used, 20 cm³ of the Yeast solution were used and 20 cm³ of the Phosphate

Buffer solution were used and these amounts were kept constant throughout the investigation.

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Before checking the volume of Carbon Dioxide collected, the mixture was always swirled once and this was maintained throughout the investigation because excessive swirling could impede the syringe and distort the results. Due to this, the mixture was only swirled once and this was always before checking the syringe.

Overall, the idea that was adopted was that everything but the carbohydrate substrate had to remain constant throughout the investigation.

The idea of including a control was avoided due to the nature of the investigation. That is, an extreme set of results could have been collected and then these could have been used for analysing the results obtained. However, since it was essential to include a carbohydrate substrate, it was not possible to have a control where only yeast (alone) is used. If temperature was investigated, then a control could be one that is not subjected to a water bath and is kept at room temperature.

Modifications.

The procedure that was carried out varied greatly to the one that was initially planned and the necessary modifications were made.

The first modification that had to be made was related to the yeast solution. Originally it had been planned that 6 grams of yeast would be added to the Glucose solution, however this proved to be a problem in more than one way. That is, this would have not been the most economical decision and also scientifically it resulted in an extremely slow reaction rate. That is, the first sign of Carbon Dioxide came thirty minutes after adding the yeast and this was too slow. Nevertheless, the yeast solution was the alternative method and this worked much more efficiently and so this modification was applied throughout the investigation.

Furthermore, since there was a carbohydrate substrate solution and a yeast solution, it had to be decided, to what ratio the both must be added. After many tests the most reliable ratio was a one to one, that is, 20cm³ of the yeast solution was added to 20 cm³ carbohydrate substrate solution.

Moreover, in the initial plan it was not decided how much of the Phosphate Buffer Solution (pH 7) was to be added and it was decided that the ratio would be the same as the above, therefore 20 cm³ of the Phosphate Buffer Solution was added.

The other modification that became indispensable was related to the amount of Paraffin that was added. Formerly it was planned that the Paraffin layer would be made using 5 cm³ of Paraffin, however this layer was too thick and no Carbon Dioxide was being collected in the syringe. Therefore, it was decided that half of the original amount would be added, that is 2.5 cm³ and this produced “fair and true” results.

Another minor modification that had to be made in the method was the fact that the mixture has to be swirled regularly as this helped increase the reaction rate. Before, the mixture would stand still and produced results after long intervals and this was too unrealistic.

An additional modification that was made was concerned with the carbohydrate substrate Mannose because this substrate was to be used in the investigation but due to lack of time, it was not possible to test five substrates. Therefore, it was decided that Mannose would not be tested, this is because background research (such as Biology “A Functional Approach by M V Roberts and T J King) suggested that this is an unreliable substrate and also it was the most expensive substrate to use.

Apart from these, not many other modifications were made, however it became clear that anti-bumping granules were not required as the mixture

did not froth much. Furthermore, a mortar and pestle were not required as the yeast dissolved quite well and the yeast did not need to be crushed.

After making these changes, the whole investigation proceeded without any problems.

Results.

A Table to Show the Results Obtained Using Glucose.

Carbohydrate	Volume Of CO ₂ (ml) Collected After"	Substrate	0 mins	5 mins	10 mins	15 mins	20 mins	25 mins	30 mins
Glucose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucose	0.0	13.5	22.0	40.0	78.5	98.5	100+		
Glucose	0.0	7.0	24.0	36.0	73.5	95.0	100+		

A Table to Show the Results Obtained Using Fructose.

Carbohydrate	Volume Of CO ₂ (ml) Collected After"	Substrate	0 mins	5 mins	10 mins	15 mins	20 mins	25 mins	30 mins
Fructose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fructose	0.0	12.0	27.5	36.5	49.5	78.0	96.5		
Fructose	0.0	13.5	29.0	46.0	46.5	47.5	49.0		
Fructose	0.0	16.5	36.0	49.0	69.5	79.5	98.5		

A Table to Show the Results Obtained Using Galactose.

Carbohydrate	Volume Of CO ₂ (ml) Collected After"	Substrate	0 mins	5 mins	10 mins	15 mins	20 mins	25 mins	30 mins
Galactose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Galactose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

A Table to Show the Results Obtained Using Sorbose.

Carbohydrate Volume Of CO₂ (ml) Collected After”| Substrate 0 mins 5 mins 10 mins 15 mins 20 mins 25 mins 30 mins Sorbose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 Sorbose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 The Average Results of all Carbohydrate Substrates used.

This set of results is the most important for analysing because they give an overall view of the data collected. Nevertheless, to make sure that the data presented was “‘fair and true’, only the most reliable results were used and so any anomalous results were not involved in this set of results. Moreover, only two sets of results were used for each carbohydrate substrate. That is, for Glucose the following two sets of results were used to calculate an average set of results.

Volume Of CO₂ (ml) Collected After”| 0 mins 5 mins 10 mins 15 mins 20 mins 25 mins 30 mins 0. 0 13. 5 22. 0 40. 0 78. 5 98. 5 100+ 0. 0 7. 0 24. 0 36. 0 73. 5 95. 0 100+ After deciding which sets of results were to be used, the next step was to calculate the averages. That is, to calculate the average volume of Carbon Dioxide collected after five minutes using Glucose, the following must be carried out: Average = $(13.5 + 7.0) \div 2$ Average = 20.5 $\div 2$ Average result for Glucose after 5 minutes = 10.3 millilitres The above method was then carried out to calculate all the average results for every carbohydrate substrate used.

A Table to Show the Average Results of all Carbohydrate Substrates used.

Carbohydrate Volume Of CO₂ (ml) Collected After”| Substrate 0 mins 5 mins 10 mins 15 mins 20 mins 25 mins 30 mins Glucose 0. 0 10. 3 23. 0 38. 0 76.

0 96. 8 100. 0+ Galactose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 Fructose 0. 0 14. 3
31. 8 42. 8 59. 5 78. 8 97. 5 Sorbose 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 0. 0 The Rate
of Reaction.

The rate of reaction of all the carbohydrate substrates could have been calculated using several methods, however, the most dependable method for this investigation is the one that is explained below.

Since, Galactose and Sorbose did not react, the only carbohydrate substrates that were involved were Glucose and Fructose.

It was decided that a table should be drawn up that would shown how much Carbon Dioxide was exactly after every interval and to calculate this, one number had to be subtracted from the other. That is, to calculate the amount of Carbon Dioxide produced between 10 and 15 minutes, the value for 15 minutes would be subtracted from the value for 10 minutes.

For example, Amount of CO₂ collected = 76. 0 – 38. 0 Amount of CO₂ collected = 38 millilitres A Table to Show the Amount of Carbon Dioxide Produced after Every Five Minute Interval for Glucose and Fructose.

Carbohydrate	Volume Of CO ₂ (ml) Collected After	Substrate	0 mins	5 mins	10 mins	15 mins	20 mins	25 mins	30 mins
Glucose	0. 0	10. 3	12. 7	15. 0	38. 0	20. 8	3. 2		
Fructose	0. 0	14. 3	17. 5	11. 0	16. 7	19. 3	18. 7		

Discussion.

The purpose of this investigation was to discover the rate of Carbon Dioxide produced by using yeast under anaerobic condition in different carbohydrate

substrate solutions such as Glucose solution as well as Fructose, Galactose and Sorbose.

The pattern of the results clearly showed that under thirty minutes Glucose produced the highest amount of Carbon Dioxide with Fructose producing the second highest amount of Carbon Dioxide under thirty minutes. However, Galactose and Sorbose did not produce any Carbon Dioxide at all in the period of thirty minutes. Nevertheless, the data clearly indicates that, in terms of producing the highest amount of Carbon Dioxide at every five-minute interval, neither Fructose nor Glucose managed to hold a steady rate. That is, at some points Fructose producing the highest amount of Carbon Dioxide and at other points it was Glucose.

Moving on, Fig. 1 and Fig. 7 clearly that Glucose was able to produce over hundred millilitres of Carbon Dioxide in thirty minutes. Fig. 7 shows a distinctive sigmoid curve and this curve indicates that a steady amount of Carbon Dioxide was produced for the first ten and the last five minutes. This further supported by Fig. 6, together "" both pieces data indicate large leaps in the amount of Carbon Dioxide collected between ten and twenty-five minutes.

Often when Glucose enters a new system, the system has to first recognise the Glucose and if it is necessary the Glucose may even be modified. An example of this is when Glucose is oxidised (after it enters a cell); it has to be modified by the addition of a phosphate group. However, in this case, as soon as the anaerobic conditions became active, the system had to

recognise the Glucose. The yeast cells slowly recognised the Glucose and started to feed on the substrate using their enzymes. This rate of reaction increased as the system had regulated with the Glucose and so, the yeast enzymes could now rapidly break down the substrate down into Carbon Dioxide and hence, creating the large leaps that were observed.

Furthermore, the drop in the Carbon Dioxide after twenty-five minutes was due to the reason that most of Glucose had been used and the amount of Carbon Dioxide that could be produced was limited.

Looking at Fig. 2 and Fig. 8, an obvious trend can be observed and that is, the amount of Carbon Dioxide being produced using Fructose is generally proportional to time. Therefore, as time moves on there is a steady increase in the amount of Carbon Dioxide being produced. Fig. 6 shows a blatant similarity in the amount of Carbon Dioxide being produced at every five-minute interval. That is, the activity of the yeast enzymes was at a steady tempo throughout the thirty minutes. On several occasions, Fructose has to be recognised and then the activity can occur. However, in this case, the system regulated with the Fructose and then the reaction proceeded at a steady rate. Therefore, a steady amount of Carbon Dioxide was being produced throughout the thirty minutes.

Fig. 3 and Fig. 4 indicate that Galactose and Sorbose did not produce any Carbon Dioxide at all. At first, it was suspected that the apparatus or the method might have been at fault. However, many different tests including repeated test using the unreactive substrates and also tests using reactive substrates (Glucose and Fructose) were carried. These tests indicated that

Galactose and Sorbose will not react the yeast used (Baker's Yeast). The unreactive substrates followed the idea that some substrates will not being able to interact with the enzymes provided by yeast.

Moreover, Fig. 5 and Fig. 9 supported the idea that even though Glucose and Fructose have the same molecular formulae, their different structural formulae resulted in different types of reactions. On the one hand, Glucose slowly regulated with the system and once the substrate was recognised the reaction carried itself on a smooth and rapid speed. With the reaction finishing with the Glucose producing over hundred millilitres.

On the other hand, Fructose managed to regulate with the system in a short amount of time and once it had, the activity of the substrate with yeast progressed at a steady level. With the reaction ending after thirty minutes and 97. 5 millilitres (on an average) of Carbon Dioxide were produced.

Fig. 6 and Fig. 10 are also very important when considering trends. They show the amount of Carbon Dioxide that was produced (on an average) at every five-minute interval. Moreover, the hypothesis was concerned with the amount of Carbon Dioxide being produced at every five-minute interval. Therefore, it is vital that these sets of data are taken into account.

Looking at the data, it is clear that at every five-minute interval for ten minutes, Fructose produced the highest amount of Carbon Dioxide but after fifteen minutes, at every interval Glucose produced the higher amount of Carbon Dioxide. At one point (at the twenty-minute interval) the amount of Carbon Dioxide produced by Glucose was more than double the amount of

Carbon Dioxide produced by Fructose. It is not till the last interval that Fructose starts producing more Carbon dioxide than Glucose. However, it must be remembered that the data used never actually gave a precise number for the thirtieth minute for glucose as Fig. 1 stated 100+ millilitres and so the accuracy of this particular data is limited.

Nevertheless, the reasoning to the trend observed in Fig. 6 and Fig. 10 remains the same as the reasoning mentioned before. That is, Fructose managed to regulate with the system rapidly and maintained a steady rate of activity. Whereas, Glucose took longer to regulate but once it had regulated, the reaction advanced at a rapid rate.

To explain the trends further, the structural formulae of the substrates needs to be discussed.

Fig. 11.

From the diagram, it is clear that even though they have the same molecular formulae, they differ in the positions of their Hydrogen and Oxygen atoms and Hydroxide groups, in relation to the Carbon atoms. This variance led to the difference in the effect of the substrate on the yeast and the overall trends in the data was also due to the different structures of the substrates.

During fermentation, the substrate is broken down (using enzymes "" to catalyse the reaction) to ethanol and Carbon Dioxide. Moreover, it needs to be discussed how fermentation occurs. The first step involves removing the Hydrogen atoms from the substrate and this is achieved by using Hydrogen

carriers such as NAD. After the Hydrogen atoms had been removed, two molecules of pyruvate ($C_3H_4O_3$) remain. From the pyruvate, another two Hydrogen atoms are removed and also, two molecules of Carbon Dioxide are removed and all that remains is "" C_2H_5OH , ethanol. In addition to this, enzymes provided by yeast would be used to break down the substrate into the products.

The rate of Carbon Dioxide production varied not only because of the system but also because of the structure of the substrate and the action of the enzymes.

Below, is a diagram of the structure of Glucose with labelled Carbon atoms: Considering that the enzymes will work based on accessibility, that is breaking down C1 first then C2 followed by C3, the pattern of the results become clearer. This is because the first Carbon atom is the most accessible and then the second Carbon atom followed by the third Carbon atom and so on. According to that, Fig. 11 clearly shows that the most accessible elements on the Glucose are the Carbon and Oxygen atoms and so, this is one of the reasons why a high amount of Carbon Dioxide is produced using Glucose.

On the other hand, Fig. 11 shows that Fructose has the Carbon double bonded on the second the carbon atom and so it is not as accessible. However, it must be remembered that first Hydrogen atoms have to be removed and then the Carbon Dioxide can be produced and so, in the light of this Fructose has a more advantageous structure. This is because the first

step of the fermentation is able to occur rapidly with Fructose, as the Hydrogen atoms are the most accessible. Moreover, then the Carbon Dioxide that is to be produced is also very accessible. The steady rate of Carbon Dioxide production using Fructose can be explained using the above idea. Looking at the data, it is clear that Fructose regulated with the system rapidly and this is because the sequence of fermentation complimented the sequence of the Fructose structure.

However, Glucose did not have a steady rate of Carbon Dioxide production and this is due to the fact that, the Hydrogen atoms that were to be removed first were the least accessible. Taking this idea in account together with the idea that the system has to recognise the substrate and then interact with it, the pattern of Glucose producing low amount of Carbon Dioxide for the first fifteen minutes can be explained. After Glucose is recognised, the Hydrogen atoms have to be removed and then the Carbon Dioxide can be produced. Taking into account all that is stated above, it is clear that this all will contribute to the slow rate of Carbon Dioxide production. However, the Carbon Dioxide being produced by Glucose did increase and this is because once the system had regulated, the Hydrogen atoms could easily be removed, splitting the substrate into two molecules of pyruvate and after that the Carbon Dioxide was easily accessible. Therefore, an increase in the Carbon Dioxide was observed and since, the Carbon and Oxygen were more accessible in the Glucose, a high amount of Carbon Dioxide could be produced.

The explanation to why Galactose and Sorbose were unable to produce Carbon Dioxide lies within the structure of the substrates. That is, these substrates did not have the correct structure to fit the enzyme “lock.’ This is because fermentation is an enzyme catalysed reaction and so the role of the enzymes is very important. That is, the enzymes provided by yeast will be able to break down the substrate into the products desired. Glucose and Fructose are naturally occurring substrates as they found in fruits as well as honey and even in the blood. Yeast is found in soils and on plant surfaces and fruits. According to this, since the yeast has developed near where Fructose and Glucose, it is likely that yeast has evolved the enzymes that are required to break down Glucose and Fructose. However, Galactose is generally prepared from lactose and Sorbose is obtainable from polysaccharides, in any case, these two substrates have not evolved near yeast and so yeast has not acquired the enzymes that required to break down the two substrates.

Moving back to limitations and inaccuracies of the investigation, it was clear from the data that some inaccuracies had taken place. Inaccuracies tend to occur due to restrictions, i. e. limitations in the apparatus, the technique used and often being restricted due to the limited amount of time.

The factor of time is a common complaint of a scientist because a limited amount of time is given to carry out the investigation and so not all the techniques used tend to be accurate. Therefore, if one had more time, the investigation is analysed and suggestions are made on improvements that can be made. There are many ways by which having more time can help

improve the overall investigation. If more was available, the first step that would be taken is that, rather than using two sets of results, the test would be repeated several times and so a larger set of results can be used. That is, more data will be available and so the conclusion drawn will be precise and moreover, the average calculated will be more reliable.

Having more time can help one extend their investigation, i. e. had more time been at hand the investigation could have been extended and so, more substrates could have been tested. In that view, Mannose could have been tested as well as unusual substrates such as Arabinose could be used. Using such substrates provides a greater deal of data and this can be used to support the ideas of the investigation.

Nevertheless, if more time had been present then the substrate solution after it had been boiled and cooled, would be placed in a system to maintain the solution's temperature at 25°C. This is due to the fact that, some fluctuations (due to the room temperature) could have occurred. Even though, the fluctuations would be minor, the accuracy of the data is in question. Besides, scientifically the most accurate data are desired and so every detail must be given attention.

One great restriction in the technique was the colour change of the Janus Green B. In this case, one had to rely on eyesight to view the colour change from blue to pink. However, this is not the most accurate way of judging the colour change and it must be remembered that the opinion (to when the “full” colour change occurred) could have varied every time that section of

the procedure was carried out. If more time was available, a colour standard could have been used. That is, to judge when the actual “full’ colour change has taken place, the colour of the “test’ solution would be compared to the colour of the colour standard, that is, the pink colour that is desired is present in the colour standard.

Apart from the technique, some of the apparatus used were restricted. The main apparatus that was limited was the glass syringe as it only measured up to one hundred millilitres and so data such as the one present in Fig. 6 and Fig. 10 was not accurate. That is, rather than stating a precise number, 3.2+ millilitres had to be stated and this limits the amount of information that can be used to draw a valid conclusion.

Moreover, the degree of accuracy could have been improved if the thermometer and the glass syringe were more precise. That is, if the temperature could have been read to the nearest decimal place, i. e. the temperature could be stated to one decimal place. This would have been very beneficial as judging when the substrate solution had reached 25oC could have been easier and the decision taken would have been more precise. Furthermore, if the glass syringe could measure volume to two decimal places, the data would have presented a higher degree of accuracy.

Moving on from inaccuracy and limitations, it is important to discuss any unusual results that may have been observed. In this investigation, some anomalous results did appear.

The first unusual result to be discussed is related to Fructose, Fig. 2 shows that the third test performed using Fructose did not advance as planned. After the fifteenth minute, a very small amount of Carbon Dioxide was being produced. That is, between the fifteenth and the thirtieth minute, only three millilitres of Carbon Dioxide was produced. The moment this observation was made, it was known that a problem had occurred (either with the apparatus or the technique that was being used). This is because the pattern of the results produced by Fructose clearly showed that Fructose is able to produce a higher amount of Carbon Dioxide in thirty minutes, this was further supported by background research and a repeated test.

The most likely reasoning available for this anomalous result is that there was a fault with the apparatus. For example, the cork may not have been tight enough on the conical or the delivery tube was not attached properly to the cork, or it could be that the glass syringe was too tight around that area.

Another anomalous result that was noted was again related to Fructose, Fig. 6 and Fig. 8 show that between ten and fifteen minutes, an uncharacteristic low amount of Carbon Dioxide was produced. This could have occurred because the flask was not swirled with equal action (before the volume was checked) and so the Fructose was unequally distributed. This would mean that the enzyme would not be able to interact at the rate it is able to because not all the substrate was available to the yeast.

Overall, not many unusual results were observed, that is, a clear pattern emerged in the data and most of the results followed the nature of the trend.

When analysing the results, the most important section that needs to be discussed is whether the hypothesis was correct or not. The hypothesis that was formulated and which was being tested throughout the investigation believed that Glucose had the ability to produce the highest amount of Carbon Dioxide at every five-minute interval. However, this was not always the case because even though, Glucose managed to produce the highest amount of Carbon Dioxide in the thirty minutes, the high rate of Carbon Dioxide production did not follow a steady trend. That is, at some intervals Fructose was able to produce more Carbon Dioxide than Glucose.

Initially, it was believed that Glucose was the most efficient substrate out of all the carbohydrate substrates that were tested. This being based on the fact that many scientists preferred using Glucose (when investigation fermentation) and furthermore, the idea that the structure of Glucose is the most capable of reacting with yeast. That is, using the enzyme “lock and key’ theory it was believed that Glucose was the most able “key’ to fit the enzyme “lock’. However, looking at the data, it is now known that this was not always the case. That is, the position of the elements on the six Carbon atoms (in the substrate) played a key role in the production of the Carbon Dioxide.

In addition, the null hypothesis that was brought into the investigation was not exactly incorrect but it was inaccurate. Since, out of the five substrates only four could be tested, from these two substrates managed to produce a fair deal of Carbon Dioxide. However, the other two followed the null hypothesis and so did not produce any Carbon Dioxide at all. This was based

on the idea that enzymes are highly specific and so the active site of the enzymes may not be able to interact with the substrate.

Overall, the conclusion that is drawn is that the amount of Carbon Dioxide produced at every five-minute interval depends on the position of the Hydrogen and Oxygen atoms as well as the position of the Hydroxide group. The accessibility of these elements enables a trend to be created in the results. Therefore, it is not possible to choose a substrate and state that it will maintain a steady rate of high Carbon Dioxide production throughout the reaction. Since, the variance in the structure will vary the rate of Carbon Dioxide being produced. However, once the system has regulated with the substrate, then it is possible to predict a clear trend and on that basis Glucose would have produced the highest amount of Carbon Dioxide.

In terms of the biological view of the investigation, one aspect of the investigation that is important to living things is the fact that yeast is only able to break down those substrates that it has evolved near. That is, the enzymes developed by yeast are able to interact with these substrates. Yeast cells rely heavily on carbohydrate substrates for energy. However, Galactose (for example) is related to milk and yeast has evolved the enzymes needed to break down this substrate.

After the system has recognised the substrate, the breakdown of the substrate can begin and this depends on its structure. That is, the position where the two hydrogen atoms are, is the first point that needs to be considered and if the atoms are accessible then they will be broken quickly.

After the Hydrogen atoms have been taken, the substrate will break into two molecules of pyruvate and then the accessibility of the Hydrogen atoms and Carbon and Oxygen atoms needs to be considered. In terms of the anaerobic respiration in yeast, the process may rapid but it is complicated and the speed at which it occurs relies predominantly on the structure of the substrate.

Further Studies.

If the investigation was to be extended then a variety of yeast would be used because it may be the case, that Galactose is able to react with Brewer's yeast but not Baker's yeast. Another extension would be to use a greater number of substrates because the conclusion that has been drawn could be further support. One other way to extend the investigation is to vary the concentration of the substrate and observe if the production of Carbon Dioxide is equally efficient.

Further studies are done to support the conclusion that is drawn and even though the conclusion that has been drawn is valid, an extended study can help support it.