Effects of enzyme efficiency | hydrolysis of sucrose

In this experiment we hypothesized if a 6ml volume of supernatant invertase is added in varying dilutions to a 2 ml volume of 1% sucrose solution, then the concentration of the end product, of glucose will be directly proportionate to the amount of invertase present. To test this hypothesis we tested varying dilutions (100%, 50%, 25%, 12. 5%, and 6. 25%) of invertase supernatant solutions. We tested using two controls the first control was used as a baseline to determine if glucose was present in the invertase supernatant without the introduction of sucrose. The second control was used to validate that glucose would not be produced without the introduction of the invertase enzyme. Benedict’s solution was used to detect the presence of glucose in the end product. Our results did not support our hypothesis, however they did show an exponential decay in the presence of glucose as the concentration of invertase was decreased. The 25% dilution of invertase showed the most efficient gain in the product glucose.

invertaseHomeostasis is a necessary characteristic of life. Homeostasis is achieved by means of self-regulating mechanisms within a cell or organism that maintain internal stability and metabolic efficiency. Enzymes are cell metabolizers that follow a metabolic pathway breaking down or building substrates for usable energy for the cell, itself, or heterotrophic organisms. The more efficient an enzyme is, the more efficiently it will produce usable products. An enzyme’s efficiency is affected by three major factors, substrate concentration, temperature, and pH levels. Under the right conditions an enzyme can be continuously reused to catalyze metabolic reactions. An enzyme’s function is defined by its R groups (structure).

In our experiment we will be using the enzyme, Invertase (beta-fructofuranosidase), a hydrolytic digestive enzyme, to catabolize sucrose to produce fructose and glucose. We will be testing the efficiency of the enzyme using varying dilutions of invertase supernatant. In order to maximize the efficiency of the enzyme, we will be homogenizing baker's yeast, disrupting the homeostasis of the organism. This process will " free" the enzyme to hydrolyze proteins, lipids, and other substrates within the internal environment, as well as, the external environment of the cells in the supernatant. The supernatant consists of an emulsified mixture of the phospholipid bilayer fragments, organelle fragments, and the " freed" enzyme. The cellulose precipitate (most dense) will be discarded. In our test groups we will be introducing 2ml of 1% sucrose solution; also, keeping the factors of heat and pH at a constant. The invertase supernatant concentration is our independent variable. Our control group of 100% invertase, without the addition of the substrate, sucrose, will show how much glucose and fructose are produced in the supernatant, itself. The degradation of sucrose will produce fairly equal amounts of fructose and glucose. We predicted that the amount of glucose and fructose produced will be proportionate to the concentration of the invertase supernatant.

There is value in degrading sucrose with the most cost effective and efficient use of invertase to produce glucose and fructose. The enzyme can continuously be reused; therefore, it can continuously produce glucose and fructose by hydrolyzing sucrose. The enzyme, Invertase, can be found in the micro-organism, yeast (Baker's Yeast) and in the microvilli of the intestine. Baker's yeast is easily obtainable and is the most cost effective choice for

industrial use. The products glucose and fructose have confectionary, food, and pharmaceutical applications in industry. Fructose's application in the confectionary and food industry produces a sweeter tasting product than table sugar and, therefore, the amount needed in a recipe is reduced. Fructose is, also, an ideal supplement in a diabetic's diet. It has a minimal effect on blood glucose levels and the secretion of insulin. The manufacturing of fructose is of great value to the food and confectionary industries. The manufacturing or production of glucose is invaluable to the pharmaceutical industry; it is used in intravenous injectable devices for diabetes patients suffering from hypoglycemia (a blood condition of a deficient amount of glucose) to stabilize sugar levels.

Our results show an exponential decay of glucose presence from higher to lower concentrations of invertase. The most glucose produced with the least concentration of invertase was our 25% dilution; it was the most efficient and cost effective result.

HYPOTHESIS: If invertase is added in varying dilutions to a 2 ml volume of the substrate Sucrose, then the concentration of the end product, glucose, will be directly proportionate to the amount of invertase present.

PREDICTION: Below is a graphical representation of our prediction based on our hypothesis.

Figure 2

MATERIALS:

Goggles and apron

Gloves

20 ml stock solution of the enzyme invertase

15 ml sucrose solution (1%)

MATERIALS cont.:

15 ml Benedict’s solution

35 ml distilled water

(20) Test tubes

(2) Pair of tongs

Waterproof marker

(2) Test tube rack

(4) 50-ml beakers

(1) Large Pipette pump

(1) Small pipette pump

(2) Large pipette tip

(2) Small pipette tip

(2) 500 ml beakers for boiling water baths

(2) Hot plates for boiling water bath

Laptop computer

Digital camera

Graph paper & colored pencils

Paper towels and disinfectant

Wash basin for used glassware

PROCEDURE:

SAFETY FIRST: Acquire goggles and aprons.

Set up the 37° C water bath incubators. Plug in the two hotplates and turn them on. Fill the two 500 ml beakers with 350 ml of water. Leave the hotplates sit until they are at a rolling boil.

Gather and organize all materials that are needed for the experiment.

Acquire the 20ml of stock solution from Professor Olsen. Pour the stock solution (invertase) into a 50-ml beaker and label it stock solution. Obtain and pour 15 ml of (1%) sucrose solution into a 50-ml beaker and label it “ S” for Sucrose. Obtain and pour 15 ml of Benedict’s solution into a 50-ml beaker and label it “ B” for Benedict. Obtain and pour 32 ml of distilled water into a 50-ml beaker and label it “ W” for Water.

Set up a “ treatment” group for the experiment by preparing a 4 step dilution of the stock solution. Acquire 6 test tubes and label them: 100%, 50%, 25%, 12. 5%, 6. 25%, and D. Place 6 ml of 100% stock solution (invertase) in the test tube labeled 100%. In test tube D, combine 6ml of 100% solution with 6 ml of distilled water. With the large pipette, pump 6 ml of this mixture in a test tube to be used as the 50% dilution in the test tube

labeled 50%. Combine 6 ml of distilled water with the remaining 6 ml of the 50% dilution. With the large pipette, pump 6 ml of this mixture in a test tube to be used as the 25% dilution in the test tube labeled 25%. Combine 6 ml of distilled water with the 6ml of the 25% dilution. With the large pipette, pump 6 ml of this mixture in a test tube to be used as the 12. 5% dilution in the test tube labeled 12. 5%. Combine 6 ml of distilled water with 6 ml of the 12. 5% dilution. Use 6 ml of this mixture as the 6. 25% dilution in the test tube labeled. Discard the remaining 6 ml of the 6. 25% dilution from the test tube labeled D.

Obtain 5 test tubes and using the small pipette, pump 2 ml of sucrose in each test tube.

NOTE: Step #6 and Step #7 below should be done simultaneously.

Prepare controls for the experiment. For the first control, with the large pipette, pump 6 ml of 100% stock solution in a test tube and label it C1 for Control One. Treat this test tube exactly as the experimental tubes are treated, EXCEPT add 2 ml of distilled water instead of the sucrose. By adding a neutral substance it will keep the volume in the control tube consistent with the experimental tubes. It is predicted that this control will show a negative reaction or otherwise stated as no change. For the second control, with the small pipette, pump 2 ml of sucrose in a test tube and label it C2 for Control Two. Treat this test tube exactly as the experimental tubes are treated, EXCEPT add 6 ml of distilled water with the large pipette instead of the stock solution. By adding a neutral substance it will keep the volume in the control tube consistent with the experimental tubes. It is predicted that

this control will show a negative reaction. No glucose is expected to be present in the control test tube since no invertase is added.

Combine the test tubes of various concentrations of stock solution prepared in step #4 above with the test tubes of sucrose solution prepared in step #5 above.

Allow both the test group (treatment group) and the control group to stand for 8 minutes.

Use the Benedict’s test to determine the presence of glucose. Acquire 7 test tubes and with the small pipette, pump 2 ml of Benedict’s Solution in each of the test tubes to be used for both the experimental and control. Once the experimental group and control test tubes have sat for 8 minutes, pour one test tube of the 2ml of Benedicts Solution from the staged test tubes into each of the experimental and control test tubes.

CAUTION: EYE PROTECTION AND APRON” S MUST BE WORN WHENEVER TEST TUBES ARE HEATED. Tongs are used to handle hot test tubes. Leave hot water in the water baths on the hotplate until cool. DO NOT CARRY BEAKERS OF HOT WATER.

NOTE: A water bath must be at a rolling boil before receiving test tubes.

Combine the test tubes of various concentrations of stock solution prepared in step #4 above with the test tubes of sucrose solution prepared in step #5 above.

IMMEDIATELY heat in boiling water for 3 minutes. Remove from the heat with tongs and place in test tube holder for evaluation. IMMEDIATELY evaluate and record the color changes.

The more glucose produced the greater the color change following the Benedict’s test.

Prepare a data chart and record the results. Each member of the team should prepare his or her own copy for reference.

RESULTS: The above experiment was performed with enzyme concentration as the independent variable and the following measurements were obtained using the color metric scale of 1 to 10 in accordance with the Benedict’s test. Our first control was a 6ml volume of 100% stock solution of invertase supernatant, which we added a 2 ml volume of distilled water to establish consistent volumes for comparison. This control measured a 5 on the color metrics scale which indicated that the enzyme invertase in the supernatant produced glucose. This control is our baseline metric to determine the amount of glucose produced beyond the supernatant. Our second control was a 2ml volume of 1% sucrose solution which we added to a 6 ml volume of distilled water to establish consistent volumes for comparison and show that glucose would not be detected without the introduction of the enzyme invertase to hydrolyze the substrate sucrose. The results were as expected, see Appendix A, Figure 5. No glucose was present in the solution.

Our experimental group consisted of 5 dilutions/concentrations of invertase to be tested. The first experimental test tube was labeled 100% stock solution; the invertase supernatant measured a 7 on the color metrics scale.

The presence of glucose did not rate as high as expected and was not proportionate to the amount of invertase concentration. The second experimental test tube labeled 50% rated a 5 on the color metrics scale. The results of glucose present were proportionate to the amount of invertase concentration. The third experimental test tube labeled 25% rated a 4. 5 on the color metrics scale. This dilution was tested 3 times to confirm accurate results. The glucose presence did not rate as low as expected and therefore was not proportionate to the amount of invertase concentration. The fourth experimental test tube labeled 12. 5% rated a 1. 25 on the color metrics scale. The presence of glucose rated as expected and was proportionate to the amount of invertase concentration. The fifth experimental test tube labeled 6. 25% rated a . 625 on the color metrics scale. The presence of glucose rated as expected and was proportionate to the amount of invertase concentration. Appendix A, Figure 4 shows a pictorial representation of the results.

The combined results of our experiment are shown in Appendix A, Figure 5. The final results, shown in Appendix A, Figure 6 and Figure 7 show the true amounts of glucose produced as a result of the experiment.

DISCUSSION:

Enzymes are catalysts and increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products.

The basic enzyme reaction can be represented by the formula in Figure 2:

Figure 3

Substrate + Enzyme Product + Enzyme

(S) (E) (P) (E)

In this equation (S) is the substrate, the substance being changed, E represents the enzyme catalyzing the reaction is the enzyme, P is the product of the reaction.

The amount of enzyme present in a reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, time, etc. The observed activity should be proportional to the amount of enzyme present and the enzyme concentration should be the only limiting factor.

This experiment concluded that the enzyme invertase had glucose present in the 100% stock solution. This was a contradiction to the original prediction that a negative response or no glucose would be present. The 100% stock solution was untreated by the independent variable; however, it still registered five on the gradient scale. This means that glucose is present in the supernatant of invertase. Therefore, in order to determine the amount of glucose produced as a result of the chemical reaction versus the amount present in the supernatant further calculations are required. When the concentration of invertase is increased, the amount of glucose produced increases proportional to the amount of enzyme concentration utilized with the exception of the 25% dilution. This suggests two possible conclusions either the maximum production of glucose occurs when the enzyme concentration is diluted to ~25% or that this was simply an anomaly in the experiment.

Our hypothesis was not supported; the amount of glucose produced was not proportionate to the concentration of the Invertase supernatant present in the solution. Our results did show an exponential decay in total glucose present as Invertase supernatant concentration was decreased.

The Invertase, enzyme, being the least dense protein in the supernatant, and acidic with a higher concentration of hydrogen ions (H+), diffuses through the supernatant from an area of higher concentration of (H+) to an area of lower concentration of (H+) to degrade the substrates (more dense) present in the solution.

Test group 1, 100% concentration of Invertase supernatant (InvS), was not as efficient as expected; therefore did not have as many effective collisions with the substrate sucrose. This is known because all other factors affecting enzyme efficiency, such as, temperature, pH, and time for the reaction, were kept at a constant during the experiment. Due to the high concentration of the enzyme in the 100% concentration of InvS there was not sufficient space within the solution to collide as efficiently with the substrate, sucrose, molecules. The 100% concentration of InvS, also, did not have the addition of water, for dilution, to aid the hydrolysis reaction. The enzyme, Invertase (Inv), weakens the hydrogen bonds of sucrose to “ break” the oxygen bridge joining glucose and fructose. The water (H2O) in the solution aids in this reaction by bonding the (H) and (OH) to fructose and glucose to complete the separation of the products. Lab group 1, also, had similar results with their 100% concentration InvS test group.

Test group 2, 50% concentration of InvS, did rate as expected; however, it was more efficient than the Test group 1 (100% concentration InvS), and less efficient than Test group 3 (25% concentration InvS), which was not expected. This result is, also, due to the amount of effective collisions of the enzyme, Inv, with the sucrose, substrate. The dilution of the Inv, making it a 50% concentration of InvS, made the 50% solution more efficient than the 100% concentration of InvS. The enzyme, Inv, diffused along the H+ gradient and had more space within the solution to have more effective collisions with the sucrose, to produce glucose. There was, also, more H2O present to aid in the hydrolysis reaction.

Test group 3, 25% concentration of InvS, proved to be the most efficient in producing glucose. Therefore, had the most effective collisions between the enzyme, Inv, and the substrate, sucrose. This is known because of the amount of glucose produced by the degradation of the added 2ml of 1% sucrose solution by the enzyme was the highest amount produced out of all of our test groups. We were able to calculate this by subtracting the amount of glucose produced in the supernatant, alone, (Control group 1((100% InvS, no added sucrose, gave us the initial amount; (as the InvS was diluted by 50%, the amount of glucose produced by the InvS was, also, reduced by 50%))) from the total glucose produced. This calculated the amount of glucose produced by the degradation of the addition of the 2ml of 1% sucrose solution by the enzyme, Inv. We are certain of our results with this test group, because we retested 3 times with the same results, each time. In the 25% concentration of InvS there is still enough of the enzyme present in the solution to have the most effective collisions with the substrate, sucrose,

present and the most sufficient room in the solution for those collisions to occur. The heat factor, in addition, aids in these effective collisions, as it did in the other groups. The active sites of the enzyme, Inv, are most efficiently used to degrade the sucrose in this solution. The increased presence of the water, also, aids in the hydrolysis reaction.

Test group 4, 12. 5% concentration of InvS, produced a proportionate amount of glucose to the enzyme concentration. It produced less than the higher concentrations, above, due to the decreased amount of the enzyme present. Even though, there was more “ space” and water present in this solution, there was not enough of the enzyme available in the solution to have more efficient collisions with the substrate, sucrose, in the solution. There was not a sufficient amount of active sites of the enzyme available to degrade the sucrose more efficiently.

Test group 5, 6. 25% concentration of InvS, produced a proportionate amount of glucose to the enzyme concentration. As with test group 4, it produced less than the higher concentrations due to the decreased amount of the enzyme present. There was not a sufficient amount of active sites of the enzyme, Inv, available to degrade the sucrose more efficiently.

Control group 1, 100% concentration of InvS without added 1% sucrose solution, did not have a negative reaction as predicted, but proved to be integral in calculating the enzymes efficiency. This group showed a presence of glucose produced by the enzyme, Inv, in the supernatant. This occurred due to the presence of proteins, lipids, and other substrates present in the supernatant from the phospholipid bilayer (cell wall) fragments and organelle

fragments of the yeast cells. We were able to use this information to calculate the amount of glucose produced in the supernatant and the amount of glucose produced by the addition of sucrose in the test groups, by subtracting it from the total glucose produced and dividing by 50% as the concentration was decreased by 50%. This showed us the true efficiency of the enzyme, Inv, degrading the substrate, sucrose.

Control group 2, 1% sucrose solution without the addition of InvS, showed a negative reaction as predicted. The reason for using this as a control was to show there would not be a reaction without the introduction of the enzyme, Inv, to degrade the sucrose, substrate. Therefore, glucose would not be produced if the enzyme, Inv, was not present in the solution.

APPENDIX A

Figure 4

Figure 4 – Results of enzyme test for the dilutions: 6. 25%, 12%, 25%, 50%, 100%

Figure 5

Figure 5 – Results of the two Control Groups:

2ml of 1% Sucrose solution combined with 6ml of distilled water

6ml Invertase combined with 2ml of distilled water

Figure 6

Figure 6: The total result of glucose present at the conclusion of the experiment

Figure 7

Figure 7: The adjusted result of glucose present as a resut of the experiment

Figure 8

Figure 8: The graphical representation of the amount of glucose prduced by the chemical reaction.