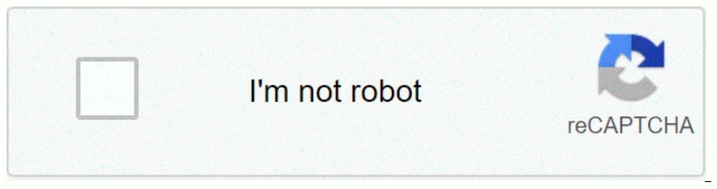

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Enzyme lab report materials and methods

Enzymes Measure 1)To understand the importance of the ratio of structure to enzyme function. 2) To be familiar with how enzymatic reactions are affected by changes in: a.Enzyme concentration b.Substrate concentration c.pH d.Ionic concentration e.Temperature f.Inhibitor (CuSO4) 3) To identify the independent and dependent variables in each of the trials A-F. Introduction In addition to your textbook, below are some web resources that will add more background information about enzymes: In each cell of a human there are many chemical reactions that take place, performing the necessary functions to be a large, complex, multicellular organism. This is relatively easy to understand. How do these reactions occur? This is not so easy to understand. Chemical reactions involve the rupture and reform of chemical bonds between molecules (substrate(s) of the reaction, which are converted into different molecules (product(s) of the reaction). Chemical reactions can occur spontaneously (without additional energy or intervention), and in fact many of the chemical reactions necessary for the life processes are spontaneous; however, some are not. Metabolic pathways are processes, involving many chemical reactions that occur in a certain order. For example, to get energy out of a molecule of glucose, a number of reactions must take place in a certain order to break the bonds between the carbons of the glucose molecule. In addition, you need to rely on a number of chemical reactions that break down stored glycogen in glucose molecules in order to have glucose molecules in the first place. If you had to rely on these reactions to take place spontaneously, you would wait a very long time - you wouldn't be here! Enzymes catalyze chemical reactions so that they occur in a timely and sequential way of producing a product. Enzymes are biological catalysts. They help to increase the frequency of chemical reactions. Enzymes are most often proteins and their three-dimensional form is important for their catalytic activity. Due to their 3D form, enzymes are very specific to the reactions they catalyze. In other words, they are very specific to substrates that they will act on. So any function, such as getting energy from a glucose molecule, actually involves many reactions, each with a specific enzyme. Enzyme activity is affected by many factors. You will examine some of the main factors, which affect the activity of an enzyme called catalase. Katalase is an enzyme, which is found in many cells, but at the highest levels of the liver because the liver often works to break down the toxins present in the blood. Catalyzes the rupture of hydrogen peroxide: 2H2O2 ------> 2H20 + O2 Peroxides can be formed in during respiration, and are chemically reactive, which means that they can chemically modify (and thus render useless) other biological molecules. Follow this link for more useful information about catalase. Materials and methods: At the laboratory table you will find everything you need to conduct today's experiments. Each laboratory station is set up to perform a different part of the Lab exercises (A-F). Contact your lab instructor for up-to-date information about which parts your group is responsible for. Activities are as follows: increasing enzyme (katalase) concentration (part A) increasing substrate (H2O2) concentration (part B) changing pH (part C) changing ionic concentration (part D) changing temperature (E) increasing concentration of enzyme inhibitor (copper sulfate) (F). The following protocol describes how to measure the activity of liver catalase by measuring the amount of O2 produced when liver catalase is combined with the substrate, hydrogen peroxide (H2O2). You will collect data in the form of production of one of the products (the volume of O2 produced in ml), and you will then convert to enzyme activity units by dividing the volume of oxygen produced by how long you allow the experiment to run. In this laboratory for easy calculation, you will measure in one minute. This will give you enzyme activity measured as a rate (ml/min). For example, after setting up the appliance as described below, you will then measure the amount of O2 collected in the graduated cylinder after one minute. If the amount of O2 produced after one minute reaction time is 32 ml, the enzyme activity is: 32 ml/1 min= 32 ml/min. For each part A-F, you want to draw enzyme activity on the Y axis (the dependent variable) and the independent variable (part A-F) on the X axis. The teacher gives you clear directions on how to prepare the entire lab report. Protocol Work as a team of 4 at your table to perform the experiment. Each group will be assigned one or more specific experiments in the laboratory. You will record your results on your teacher's computer, and these results will be made available to you as a link from the lab's website or as an email attachment to make the write-up. Each group must analyze all 6 parts (A-F) of the experiments from the lab's aggregate data, even if the group does only one or two parts. At the table you will find a small rectangular bottle equipped with a rubber stopper and metal tubes (reaction container), a 100 ml graduated cylinder and holder, a plastic pan and a supply of small pieces of filter paper. Fill the pan 2/3 full of tap water, which quickly becomes room temperature. Lower the graduated cylinder to fill it with water. Turn the graduated cylinder upside down, hold the open end underwater, and hang it upside down in the clamp. Adjust the height of the clamp so that the open end of the is about 2 cm below the surface of the water. Place a thermometer in the pan and once during part A, record the temperature of the water. Figure 1 shows an image of the layout. Figure 1: Experimental enzyme lab setup For all parts A to F, each reaction vessel will have 3 soaked disks and 10 ml substrate solutions as shown in Figure 2 below: Figure 2: Reaction container The following procedures are used to achieve oxygen production and should be repeated for all sections A-F. 1. Remove the stopper and place the reaction container on the side of the table. Then, using seaweed, dip three filter disks (one at a time) in the enzyme solution. Remove excess liquid from the disks by dabbing a corner of the filter paper on a Kimwipe or paper towel. Then the muted disks transfer to an inside wall of the reaction container. Place the disks in the front lower half of the reaction container (half the nearest opening). A person in each group should suck and handle all disks for all experiments. Such procedures should be used in group experiments to ensure that important operations are performed exactly the same way. 2.Rotate the reaction container so that the disks are on the top side (see picture above) and then add 10 ml of substrate (H2O2) solution. Be careful not to splash H2O2 on the disks. Insert the stopper (with metal tube) into the reaction container. 3.Hold the side with the disks upwards and carefully place the reaction container on the side of the pan with water. The metal pipe is located directly below the opening of the upside-down graduated cylinder. 4.Get the timer ready! Rotate the reaction 180 degrees so that the disks are covered by the H2O2 solution. This is time zero and start the timer now. 5.Measure the oxygen level in the graduated cylinder after 1 minute (from the reaction container is switched on the side). 6.Remove the disks, rinse and dry the reaction container A.INFLUENCE OF CATALASE CONCENTRATION ON TOTAL ACTIVITY Repeat steps 1-6 using various enzyme resolution: 1)Three catalase-soaked disks 2)Two catalase-soaked disks and 1 disk soaked in water 3)A catalase-soaked disk and 2 disks soaked in water 4) Three disks soaked in distilled water. The substrate for all four cases is 3% H2O2 solution. Graph your results as enzyme concentration (x-axis) vs. Speed (Vol of O2/min) and explain the relationship, which appears to exist, between the concentration of the enzyme katalase and the breakdown of the peroxide. B. INFLUENCE OF SUBSTRATE CONCENTRATION ON CATALASE ACTIVITY Repeat steps 1-6 using three cataslase-soaked disks), using different concentrations of substrate, H2O2, as shown below. 1.0% H2O2 (distilled water) 2.0.3% H2O2 3. 1.5 % H2O2 4. 3.0% H2O2. Graph the results as substrate concentration (x-axis) vs. Speed (Vol at O2/min) and explain the ratio, which appears to exist between substrate and the frequency of enzyme activity. C. INFLUENCE OF pH ON CATALASE ACTIVITY Repeat steps 1-6 with three catalase-soaked disks, using cata laser solutions at the various pH values shown below. Use 3% H2O2 as a substrate for each study. 5)pH 2 6)pH 4 7)pH 7 8)pH 10 Graph your results as pH (x-axis) vs. Speed (Vol of O2/min) and explain the relationship that appears to exist between pH and cata laser activity. Repeat steps 1-6 (using three catalase-soaked disks) using different concentrations of NaCl in the substrate solution. 1.1.5% H2O2 resolution containing 10% NaCl. 2.1.5% H2O2 resolution containing 2% NaCl. 3.1.5% H2O2 resolution containing 0% NaCl. Graph your results as ionic concentration (x-axis) vs. Speed (Vol of O2/min) and explain the relationship that appears to exist between the concentration of sodium chloride ions and catalase activity. E. CATALASE TEMPERATURE IMPACT REPEAT steps 1-6 (use three catalase-soaked disks and 10 ml 3 % H2O2) in each of the different temperature water baths. 1.Three disks soaked in boiled catalase extract (100C) in a room temperature water bath and reaction vessel with 3% H2O2. Allow the equipment to stabilize at room temperature for 2-3 minutes before running. This simulates a boiling water bath, without danger. 2.Et water baths that have been cooled with ice to 5C. (Continue adding ice to keep the temperature close to 5C. Let the reaction container stabilize in the water bath for 2-3 minutes before running. Record the temperature. 3.Et water baths heated up to 33C. Allow the temperature of the reaction container to stabilize in the water bath for 2-3 minutes at 33C before running. This temperature is about 91.4F, which in the body corresponds to a state of hypothermia. 4.Et water baths heated up to 37C. Allow the temperature of the reaction container to stabilize in the water bath for 2-3 minutes before running. This temperature is about 98.6F, which corresponds to the standard body temperature. 5.Et water baths heated up to 41.1C. Allow the temperature of the reaction container to stabilize for 2-3 minutes in the water bath before running. This temperature is about 106F, which in the body, corresponds to a state of hyperthermia. 6. Use the results of the study from Part A, step 1 (catalysis action with 3 disks at room temperature) for the room temperature data point requested on the class worksheet. Graph your results as temperature (x-axis) vs. Speed (Vol of O2 /min) and explain the ratio that seems to exist between temperature and cata laser activity. F. INFLUENCE OF ENZYME INHIBITOR ON CATALASE ACTIVITY Repeat steps 1-6 using 3 disks soaked with cataslase at different concentrations of copper sulfate. You will receive five tubes containing the cata laser solution, five beakers containing copper sulfate (CuSO4) solution at concentrations and five weighing boats. Weigh boat 1: Pour all 5 ml of catalase solution, then add 5 drops 0.1 M CuSO4 solution. Mix and wait exactly 5 mintues before making the run. Weigh boat 2: Pour all 5 ml of catalase solution, then add 5 drops 0.25 M CuSO4 solution. Mix and wait exactly 5 mintues before making the run. Weigh boat 3: Pour all 5 ml of catalase solution, then add 5 drops 0.5 M CuSO4 solution. Mix and wait exactly 5 mintues before making the run. Weigh boat 4: Pour all 5 ml of catalase solution, then add 5 drops 0.75 M CuSO4 solution. Mix and wait exactly 5 mintues before making the run. Weigh boat 5: Pour all 5 ml of catalase solution, then add 5 drops of 1.0 M CuSO4 solution. Mix and wait exactly 5 mintues before making the run. Plan your experiment carefully!!! Graph the results as copper sulfate concentration (M) (x-axis) vs. Speed (Vol of O2/min) and explain the ratio, which appears to exist between copper sulfate concentration and cata laser activity. The data point 0 M copper sulfate is the value generated in part A #1: three disks soaked in katalase. G. INSTRUCTIONS FOR ENZYME PAPER - rough draft is due week 21. As a group, complete a full scientific article. This paper will contain parts A-F, even if the group only completed part of the experiment. Here are a few internet resources that will help you write a scientific article about the experiment. Write a lab report: a link to an introductory biology course at UNCG that deals with writing a laboratory report. Writing Lab reports and scientific articles: by Warren D. Dolphin at Iowa State University Use the Long Island University library website for the correct MLA format to quote your references in the report. Dr. Garrison's sample lab report Note 2: Use the text and internet resources at the beginning of this lab to discuss the basic principles of enzyme function. Also include your background information about catalysis activity. Also in discussing your results, in the discussion section you also need to do some research to explain the effect the different conditions have on catalysis activity. REFERENCES Abramoff, P. & Thomson, R. G. 1976. An experimental approach to biology, p. 65-73. W. H. Freeman and Co. Starr, C. & Taggart, R. 1981. Biology: The Unity and Diversity of Life, p. 84-94. Wadsworth publishing house. Weisz, P. D., & Tarp, F. H. 1967. Laboratory manual for elements of biology, p. 25-32. McGraw-Hill Book Co. Back to 270 lab manual link left

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