

Manipulation of Enzymes and Enzymatic Processes

Methods and Materials

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The following description of the methods and materials for two experiments, salivary amylase and phosphorylase, can be found on pages 39 to 45 of the fall 2014 Biology 130 [Biol.130L], (Department of Biology, 2014) lab manual for the materials and procedures of this lab. All steps were followed with no deviations.

Note: The steps listed in this section have been intentionally shortened for the purposes of demonstration. An actual Lab Report would contain more accurate detail.

Salivary Amylase.

The following steps were followed to conduct the salivary amylase experiment.

Step 1: preparing test tubes and beakers. In the first step for this experiment, test tubes and beakers were labelled to ensure accurate identification. Twenty test tubes were labelled #1 through #20; two 50ml beakers were labelled #1 and #2; one 100ml beaker was labelled #3; and two 250ml beakers were labelled #4 and #5. After all equipment was labelled, water was added to the beakers by, first, filling 200ml. of tap water into beaker #5 and then transferring (with the use of measuring cylinders) the beaker #5 water into the other beakers as follows: 9ml in beaker #1, 19ml. in beaker #2, 49ml. in beaker #3, and 99ml. in beaker #4.

Step 3: administering the iodine and Benedict's tests on a starch suspension control. In test tube #5, 2 ml. of a 1% (0.25% NaCl) starch suspension was added. Using a new spot plate, the iodine test was carried out followed by the Benedict's test (as prescribed in step 3). The results of each test were recorded.

Step 4: preparing test tubes for reaction rate tests. Using a 10ml graduated cylinder, 2ml. of water from beaker #5 was placed in test tube #10. Then, 2ml. each of 1%, 2%, 5%, and 10% salivary amylase solutions were placed in test tubes #9, #8, #7, and #6, respectively. To test tubes #11-#15 were added 2ml. each of the 1% starch solution and McIlvaine's buffer (to maintain an optimal pH). Once test tubes #6-#15 were prepared, they were placed in a rack and then in a 37 degree water bath and left for 5 minutes. During this time, two spot plates were prepared by adding one drop of iodine solution to the wells.

Phosphorylase.

Step 1: pre-lab preparation. The following activities were prepared by teaching assistants for the labs. Six hundred grams of potatoes were peeled for 32 students. The potatoes were cubed and then, using a blender, the potatoes were homogenized with 400ml. of .01N sodium fluoride. The mixture was filtered through a cheesecloth and then centrifuged at high speed for 5 minutes. The mixture was given an iodine test to ensure that no starch from the potatoes was transferred with the enzyme.

Step 2: prepare test tubes. In order to assess the effects of varying concentrations of substrate and product on the enzymatic reaction, various test tubes were prepared with different combinations of substrate, reactant and product. Eight clean test tubes were labelled #1 to #8. In test tube #8 was placed 4ml. of fresh phosphorylase.

Page 5 of 12

1

Headings and subheadings help to organize the methods described in the lab.

2

Materials are introduced as each step in the method is presented.

3

Important details are included to ensure repeatability.