Simple Enzyme Experiments

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Introduction

Thousands of enzymes are found in living cells where they act as catalysts for the thousands of chemical reactions which occur. In addition to making life possible, many enzymes have numerous applications that affect our daily lives in other ways such as food processing, clinical diagnoses, sewage treatment, and the textile industry. Enzyme experiments are ideal for "hands on" opportunities and since several factors affect the rate at which enzymatic reactions proceed, an enzyme experiment presents many opportunities in the biology laboratory. The experiments presented in this chapter were selected on the basis that they: (1) are simple to prepare, (2) use materials which are familiar to the student, and (3) can be used as a base from which to construct additional experiments. Instructions are given for a basic reaction which can then be modified to investigate various aspects of enzyme activity such as the effect of temperature, enzyme concentration and substrate concentration, depending on the level at which they are used. The following enzymes are included: amylase, catalase, catecholase, invertase, papain, pectinase, pepsin, and rennin.

Except for the pepsin experiment, all experiments can be completed during a 2- to 3-hour laboratory period. The time for each individual experiment varies from "instant" results with catalase to an incubation period of 30 minutes or more with amylase. Except for the enzyme solutions, materials listed with each experiment are based on what one student will need if working alone. The enzyme solution is usually enough for a class. Most quantities are expressed in more than one way so that the experiments can be done with a minimum of equipment. For further reading see Allan et al. (1983), Mader (1982), Morholt et al. (1966), Novo Laboratories (1975), and Porter et al. (1973).

Amylase

Introduction

Amylase is an enzyme that catalyzes the hydrolysis of starch (amylose), a polysaccharide, into maltose, a disaccharide. This process is vital for energy release in germinating seeds and is also critical in human digestion. In this experiment, you will compare the activity of amylase in soaked (germinated) barley seeds, dry barley seeds, boiled barley seeds, and human saliva. The iodine solution acts as an indicator for the presence of starch, turning purple where starch remains and leaving clear zones where starch has been hydrolyzed.

The goal is to observe and measure the enzymatic activity of amylase under various conditions and quantify its effect by measuring the diameter of the clear zones where starch has been broken down.

Materials

Barley seeds (soak for 24 hours to initiate germination; additional dry and boiled seeds for comparison)
Starch-agar plates (0.2% soluble starch, 2% agar)
Iodine solution
Distilled water
Wax pencil / marker (for petri dishes)
Razor blades

Procedure

Preparation of Starch-Agar Plates:

1) Prepare starch-agar plates by dissolving 0.2% soluble starch and 2% agar in water, heating gently to dissolve.
2) Pour the mixture into Petri dishes and allow it to cool and solidify. Plates need not be sterile if used within a day or two.

Labeling Plates

Use a wax pencil to label the bottom of each starch-agar plate with different treatments:

- "Soaked seeds" (germinated barley seeds)
- "Dry seeds" (ungerminated barley seeds)
- "Boiled seeds" (heat-inactivated seeds)
- "Saliva" (optional: a few drops of human saliva)

Seed Placement and Incubation

1) Using a sharp razor blade, carefully cut barley grains longitudinally to expose the cut surface.
2) Place the seeds, cut-side down, on the agar surface. For consistency, ensure seeds are spaced at least 2 cm apart to prevent overlapping zones of hydrolysis.
3) Incubate the plates at room temperature for 30 minutes. During this time, amylase from the soaked seeds (if active) will begin breaking down the starch in the agar

Measuring

1) After incubation, gently remove the seeds from the plate and rinse the surface with distilled water to remove loose debris.
2) Flood the plate with iodine solution. Swirl gently to ensure the entire agar surface is covered, allowing the solution to react with remaining starch for 1-2 minutes.
3) Pour off excess iodine and rinse briefly with distilled water.
4) Observe the plate. Areas where starch remains will stain deep purple. Clear zones indicate starch hydrolysis by amylase activity.
5) Use a ruler to measure the diameter of the clear zones (in mm) for each treatment. Record the results.

Results

Expected Observations:

- Soaked seeds: Clear zones should form around the seed placement, indicating active amylase hydrolyzed the starch.
- Dry seeds: Minimal or no clear zones are expected, as dormant seeds produce little to no active amylase.
- Boiled seeds: No clear zones are expected because boiling denatures the amylase enzyme, rendering it inactive.
- Saliva (optional): Clear zones should form, as saliva contains active amylase.

The diameter of clear zones is proportional to amylase activity. Larger diameters suggest higher enzymatic activity. Compare the results for each treatment to understand the effect of germination, boiling, and dormancy on amylase activity.

Catalase

Introduction

Hydrogen peroxide (H_2O_2) is naturally formed in living organisms, however it is very harmful and is broken down immediately by several enzymes including catalase. This enzyme catalyses the breakdown of hydrogen peroxide to water and oxygen. Persons with acatalasemia (a hereditary condition) have extremely low catalase activity and, although present worldwide, it is more commonly found in Koreans.

Materials

Test tubes (one for each material to be tested plus extra for control)
Hydrogen peroxide (H_2O_2) (3% solution)
Assorted living tissue: sliced raw potato, ground meat, liver, yeast cells, ground young leaves
Assorted non-living material: piece of baked potato or cooked liver, etc. (Use caution with rocks or sand, some will "bubble.")

Ruler or graduated cylinder (to measure foam height in mm/cm)
Stopwatch or timer (optional, for time-based comparisons)

Procedure

1) Fill each labelled test tube approximately 1/3 full with fresh hydrogen peroxide.
2) Add a small amount of material to be tested.
3) Note whether or not bubbles are produced.
4) Record the height of the foam (in mm or cm) produced in the test tubes to quantify catalase activity.

Note: You should be able to brainstorm alternate lab techniques to measure the volume of gas produced.

Results

Any fresh, living material will normally have enough catalase present to produce bubbles of gas (oxygen) upon exposure to the hydrogen peroxide. Dry yeast and liver are most impressive when used in this experiment.

Catecholase

Introduction

The brown color, which usually develops when a potato or apple is cut or bruised, is the result of a chemical catalyzed by several enzymes. One of the most important of these enzymes is catecholase (catechol oxidase or polyphenol oxidase). This enzyme is brought into contact with its substrate, catechol, when cells are ruptured and exposure to air (oxygen) resulting in the oxidation of catechol to the brownish colored benzoquinone. This so called "wound reaction" is apparently protective since the quinones are toxic to microorganisms.

Materials

0.1% Catechol solution (in dropper bottle with dark glass)
Distilled water (150 ml plus full dropper bottle)
Electric blender

Test tubes (2)
Test tube rack
Dropper bottle for potato extract Beaker (250 ml)
Cheese cloth
Stirring rods (2)
Balance
Optional: Spectrophotometer (to measure absorbance at 420 nm).

Procedure

1) Prepare catecholase by thoroughly blending potatoes (15 g/100 ml of water) in distilled water. Filter through cheesecloth. Put filtrate in a dropper bottle.
2) Fill each of two test tubes 1/4 full (3 ml) of distilled water.
3) Add 10 drops of catechol to each tube.
4) Add 10 drops of potato extract to one tube, label C.
5) Add 10 drops of distilled water to the unlabelled test tube. Mix.
6) Note and record color of each tube at 1-minute intervals for 5–10 minutes.
7) Use a spectrophotometer to measure the absorbance of the reaction mixture at 420 nm at regular intervals (e.g., every minute for 10 minutes) to quantify the color change due to benzoquinone formation.

Results

The contents of tube C (catechol plus enzyme) will darken with time as the reaction proceeds while the control tube remains clear.

Invertase

Introduction

Our most common food sugar—the disaccharide, sucrose—is formed in all green plants. The metabolism of sucrose in the animal body begins with the action of invertase (sucrase) which hydrolyzes the disaccharide to two monosaccharides, fructose and glucose. This same enzyme is also produced by plants and fungi.

Materials

Sucrose (0.25 M solution)
Glucose (0.25 M solution)
Benedicts solution (in dropper bottle) Distilled water (100 ml)
Hot plate with water bath
Stirring rods (2)
Beaker (50 ml)
Test tubes (5)
Test tube rack
Pipet or syringe to measure 3 ml
Tes-Tape (tape from drug store used to test for glucose in urine samples)
Balance
Spectrophotometer (to quantify reducing sugars' absorbance at 600 nm).
Pipette for precise measurement of liquids.

Procedure

1) Prepare yeast by mixing 1 teaspoon (3 g) dry yeast with 20 ml of distilled water. Let stand for 20 minutes.
2) Fill each of two test tubes 1/3 full with sucrose solution.
3) Add 3 ml yeast suspension to one tube (label 1). Mix.
4) Add 3 ml distilled water to the other tube. Mix.
5) After 10 minutes test each test tube plus the yeast suspension with a strip of Tes-Tape. (Benedict's test for reducing sugars can also be used here since sucrose will give a negative Benedict's test and glucose/fructose will give a positive test (yellow-orange-red) depending on the amount of reducing sugar present. Remove about 2 ml of the solution, place in another test tube, add 10 drops of Benedict's solution and place in a boiling water bath for about 3 minutes. Prepare a glucose solution for comparison.)
6) Quantify reducing sugar concentration using Benedict's test. After the boiling water bath, measure the absorbance of the solution at 600 nm using a spectrophotometer or compare it against a standard color chart.

Results

For best results, do not go over the recommended incubation period. Both the Tes-Tape and Benedict's give positive tests for samples from tube 1 while the tube without yeast gives negative results.

Papain

Introduction

Papain, from the latex of the papaya plant, is one of a family of plant enzymes that includes bromelin (from pineapple) and ficin (from fig), all of which break down proteins. This is why the directions on a box of Jello remind you never to use fresh or frozen pineapple in your gelatin, since gelatin is the protein responsible for the “gel.” A convenient source of papain is fresh pineapple juice or meat tenderizer.

Materials

Gelatin (Knox, Jello)
Beaker (150 ml)
Balance
Stirring rods (3)
Test tubes (2)
Test tube rack
Beaker of ice water
Hot plate
Distilled water (100 ml)

Procedure

1) Prepare a gelatin solution by heating 1 teaspoon (3.0 g) of gelatin in 100 ml distilled water until dissolved. (Gently mix, do not boil.) Cool to room temperature.

2) Pour meat tenderizer into one of the two test tubes until it fills approximately 0.5 cm of the tube. Label this tube as P. Do not put meat tenderizer in the other tube.
3) Fill each test tube 1/3 full (5 ml) with the gelatin solution. Mix gently.
4) Place tubes in ice water for 10 minutes.
5) Remove from ice bath and note the degree of gelatinization.
6) Measure the viscosity of the gelatin solution with and without papain using a viscometer or record the percentage of gelatin that remains solid versus liquid after treatment.

Results

The tube without meat tenderizer (papain) will contain firm gelatin. Tube P which contains papain will be almost liquid.

Pectinase

Introduction

Pectinases are enzymes that break down the polysaccharide pectin, which is located primarily in the middle lamella of cell walls in plants. Pectin compounds are widely distributed in plant tissues, especially in fruits. They are large molecules which are responsible for holding dispersed particles in suspension in fruit juices. In addition to influencing the amount of suspended particles in a juice, pectins also increase the viscosity of the juice. The presence of suspended particles in tomato and orange juice is not undesirable to most people, however, "clear" juices such as apple and grape are more often preferred. Commercially prepared pectinase can be added to prepared fruits in order to hasten the release of juice and aid in the "settling out" of suspended particles in fruit juice.

Materials

Funnels (2)
Graduated cylinders, 100-ml (2)
Beakers, 50-ml (2)
Pipets or syringe, to measure 0.5 ml liquid (2)
Spatulas or spoons (2)
Apple sauce
Distilled water (10 ml)
Pectinase (available from several biological supply companies or businesses providing cider and/or wine making supplies)
Wax pencil
Cheesecloth (two squares to fit funnel)

Procedure

1) Place approximately 25 ml of apple sauce into each of the two beakers labelled "no enzyme" and "pectinase".
2) Add 0.5 ml distilled water to the "no enzyme" beaker and 0.5 ml pectinase to the "pectinase" beaker.
3) Stir the contents of each beaker thoroughly (using separate spatulas).
4) Let stand 10 minutes or longer.
5) Place cheesecloth in a funnel and place the funnel into the graduated cylinder.
6) With the aid of the spatula, pour contents of each beaker into a separate funnel and collect filtrate.
7) Measure the volume of juice (in mL) collected in the graduated cylinder after filtering through cheesecloth to quantify pectinase activity.

Results

The volume of filtrate from the apple sauce plus pectinase is usually at least double that of the filtrate without the enzyme.

Pepsin

Introduction

Pepsin is an enzyme which aids in the breakdown of proteins and is secreted in the stomach of most animals. It functions at a very low pH, which makes it ideal to use for studying the effect of pH on enzyme activity. Commercial or natural albumin can be used as the protein source; however hard- boiled egg white is more impressive to demonstrate the action of pepsin.

Materials

Egg white (hard-boiled) Test tubes (6)
Test tube rack

Solutions:
A: 1.0% Pepsin
B: Pepsin (1.0%) in 0.4% hydrochloric acid C: 0.4% Hydrochloric acid
D: Pepsin (1.0%) in 0.5% sodium bicarbonate E: 0.5% Sodium bicarbonate
F: Distilled water

Metric ruler Knife

Procedure

1) Label test tubes A to F.
2) Fill each tube 1/3 full (5 ml) with the corresponding solution.
3) Drop a small (2 mm) cube of egg white into each tube.
4) Incubate at room temperature for approximately 12 hours. (The speed of this reaction can be increased by using very thin strips of egg white and/or incubating at 30°C. Students might want to compare the action of papain or bromelin under similar conditions.) 5. Examine tubes for the presence of the egg white.
5) Measure the decrease in the size of the egg white cube (in mm or mg) using a ruler or balance to quantify pepsin activity over time; OR measure before/after mass change of egg white.

Results

After 12 hours, the egg white will be “digested” in tube B; little, if any, of the cube will remain, while there appears to be little change in the other tubes. If allowed to incubate longer, the cube in tube D and possibly C will decrease in size.

Rennin

Introduction

Rennin is an enzyme obtained from the fourth stomach of a calf or other young bovines. It causes rapid clotting of milk by causing certain bonds to break in the soluble casein molecule (milk protein) converting it to the insoluble casein, thus producing "curdled" milk. Most other proteolytic enzymes will curdle milk, but they also cause continued breakdown of the casein, unlike rennin. Other forms of rennin exist as rennet (an extract of the enzymes from the fourth stomach, i.e., not pure rennin) and rennilase or hannilase which are trade names for milk-clotting microbial enzymes. This enzyme is available at some health food stores and is present in Junket which is found with the home-made ice cream supplies at many grocery stores.

Materials

Rennin (or Junket tablet)
Milk (preferably non-UHT and at room temperature)
Distilled water (50 mL)
Beaker (100 mL)
Test tubes (2)
Test tube rack
Stirring rod
Stopwatch or timer
Thermometer
Water bath (optional for controlled temperature)

Procedure

1) Prepare Rennin Solution:
   - Crush one Junket tablet and dissolve it in 50 mL of distilled water.
   - Divide the solution into two equal portions.
   - Boil 25 mL to deactivate the enzyme (boiled rennin).
   - Leave the other 25 mL unboiled (active rennin).

2) Set Up Milk Samples:
   - Pour 3 mL of milk into each of two labeled test tubes:
   - Tube A: Add 3 mL of unboiled rennin solution.
   - Tube B: Add 3 mL of boiled rennin solution (as the control).

3) Mix Thoroughly:
   - Use separate stirring rods to mix the contents of each test tube to ensure uniform distribution of the enzyme.

4) Incubation:
   - Place the test tubes in a water bath or leave them at room temperature for observation.
   - If using a water bath, maintain it at approximately 37°C (optimal for enzyme activity).
   - Start the timer.

5) Observation:

- Check both tubes every minute for signs of milk clotting.
- Gently tilt the test tubes to observe whether the milk has formed a solid curd or remains liquid.

6) Record Clotting Time:
   - Record the time (in minutes and seconds) it takes for the milk in Tube A to visibly clot.
   - Note any changes in Tube B (expected to remain liquid as the enzyme is deactivated).

Expected Results

Tube A (Unboiled Rennin): Milk should clot within 5–10 minutes at room temperature or more quickly in a 37°C water bath.

Tube B (Boiled Rennin): No visible clotting is expected, as the enzyme has been denatured by boiling.

Literature Cited

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