SCIENCE & HEALTH EDUCATION PARTNERSHIP
UNIVERSITY OF CALIFORNIA · SAN FRANCISCO

Amylase Experiment 2.0

Lesson Overview

Grade level(s):

High School (9-12), Grade 9, Grade 10, Grade 11, Grade 12

Subjects(s):

Biology/Life Science, Science Skills

Topic:

enzymes, food testing, starch, experiment, experimental design

Big ideas(s):

Enzyme activity can be affected by a wide range of factors.

What you need:

Access to a standard wet chemistry-oriented lab for preparation of materials.

Other items: cornstarch, 4% iodine solution, a big bag of eppendorfs, small weigh boats or dixie cups, large filter papers

Grouping:

For the first part of this lesson, students can work in pairs.

During the experimental design phase, the students can work in groups of four. In this group, students design their own experiments to affect enzyme function.

Setting:

laboratory, classroom

Time needed:

Two classes of about 70 minutes each.

Author Name(s):
Sam Pollock, Daniel Hensley, Deb Apple

Summary:

Students will use the amylase starch digestion experiment to see enzymes in action. After they've done a run-through of the basic protocol, they'll add a variable of their choosing in a student-designed experiment and share their results.

Prerequisites for students:

- Familiarity in basic protein structure, general chemistry (pH, temperature, activation energy, elements), enzymes.

- Students must have some basic laboratory skills such as measuring, weighing, and pipetting.

Learning goals/objectives for students:

Through this lesson plan students will explore the central concepts of experimental design (controls, changing a single variable, collecting and presenting quantitative data, and planning follow up experiments).

Content background for instructor:

This lesson plan was inspired by and draws very heavily from another lesson plan found on the SEP website, "Amylase - Exploring digestion and evolution through a molecular machine". In fact, most of the concepts and materials used are the same, but *this version optimizes the starch solution concentration*, and uses a more efficient experimental set-up.

The other lesson plan does an excellent job setting the background for amylase as a topic to explore evolution, copy number, etc. The approach in this lesson plan is to teach experimental design with a simple and optimized system. For background information of this experiment, please see the original lesson plan.

There are multiple topic areas that can be explored using this experiment. i.e. evolution, enzyme chemistry, analytical chemistry, optics, experimental design, carbohydrate structure and function etc. The basic lesson plan below will be a starting point for a lesson that can be focused in on various topics.

Getting ready:

To prepare for the lesson you will need access to a science bench or lab space. The instructions below will walk you through the preparation of the lesson.

STEP 1. MAKE THE STARCH SOLUTION (Do this the night before so the solution is fresh)

To make the starch solution, add 475 mL water (we used distilled) to a 1 L beaker with a large stir bar. Place on a hot plate with slow stirring, crank up the heat, and cover loosely with aluminum foil. Bring to a boil. Meanwhile, add 625 mg cornstarch to a 50 mL falcon tube, followed by 25 mL of room temperature water. You'll get a very opaque suspension and, if you let it settle at all, will see a big glob of cornstarch at the bottom.

When the beaker water is at a vigorous, rolling boil, add the cornstarch (be sure to completely suspend the cornstarch by vortexing or inversion). Turn the heat off on the hot plate. When the beaker has cooled down enough to move with nitrile gloves, move it to the middle of the hood and let it cool to room temperature.

NOTE: In past experiments we have found that the procedure does not work well in the presence of aggregates (even if the solution is dilute). The iodine solution stains these aggregates and they are inaccessible to the amylase. To resolve this we recommend filtering the solution. Please note that the filtering process can be time consuming.

To filter, line up as many 125 mL beakers or plastic funnels as you can, whatever the limiting item is. Take a piece of large filter paper and fold such that you make a filter cone. Place in a plastic funnel and place both above a beaker. The paper will try to jump out of the funnel initially, but as soon as you pour some starch solution in it will stay put. Set up as many filter apparatuses as you can and wait, refilling as often as possible. As soon as you've filtered all the starch solution (or at least 200 mL, which was more than enough for 50+ students), pour into an appropriately sized bottle and store.

STEP 2. DILUTE THE IODINE SOLUTION (Do this the night before so solutions are fresh)

The iodine solution at the SEP resource center (Resource Center Call Number E368 or K243 or bought at a drugstore as tincture of iodine) should be at 4 %. Simply dilute this 10 X to make a 0.4 % solution. The solution must either be stored in a tube wrapped in tinfoil (i.e.15 mL falcon tubes with one per table) or in brown tubes or jars, as the iodine is *sensitive to light*.

STEP 3. COLLECT THE EPPENDORFS AND WEIGH BOATS

Get a big bag of eppendorfs (typically come in 500 tube bags) and a big stack of weigh boats (or dixie cups). Students collect their own eppendorfs (to make stock solutions) and weigh boats (to spit in) from one area of the room.

Lesson Implementation / Outline

Activity:

DAY 1: AMYLASE EXPERIMENT

BEFORE THE STUDENTS ARRIVE:

The way students will quantitative this experiment is by measuring the time it takes for their starch solution to go clear. In order to properly do so, they need an "endpoint" tube where a standard experiment has been allowed to run longer (10+ minutes) to break down most of the starch. They will compare this with their experiment tube and when they can no longer tell the difference between the two, will call time. Therefore, before the students arrive, run an experiment as described below for each groups (there should be one reference tube per table.)

NOTE: Remember that starch as a polymer in solution (in the presence of iodine) is colored but as a monomer it is not catabolized.

AFTER THE STUDENTS ARRIVE:

Demonstrate to the students how the experiment works. In a previous class (or the day of). Present to the students the background content of the experiment. As an instructor, you can decide what level of depth (in terms of background) you want to present. For this experiment, students should be familiar with pipetting and basic lab safety. You can consider adding control samples in your experiment. Be aware that some students might find the concept of a control confusing.

To complete the experiment students will follow the steps below in groups of two:

- Pipette 1 mL of the filtered starch solution into an eppendorf.
- Grab a weigh boat and spit into it (you'll need 0.2 mL per tube)

- Take the spit boat and the starch solution back to their tables and add 100 uL 0.4% iodine from the tinfoiled tube to the starch solution.

- Invert the eppendorf a few times. The color change should be dark blue/purple. Emphasize that this entire process should be done as fast as possible (since the iodine loses color and the amylase loses potency over time).

- Next, have one of the students ready a timer. After adding 0.2 mL of spit directly into the starch/iodine solution, start the timer.

- Invert the tube continuously (don't shake it!) and watch the color disappear. When they can no longer tell the difference between the tube you just prepared and the "endpoint" tube on the table, stop the timer.

- Record your result on the board.

- Discuss with the classes the similarities and differences in the data.

DAY 2: PREPARATION FOR EXPERIMENTAL DESIGN

This day is all about preparing them to run their own experiments. Optional topics for introduction include: experimental design, protein chemistry, controls, and variables. Another option that can be used in your classroom is to divide the students into "biotech study groups". In these groups ask the students to come up with a way to disrupt the function of an enzyme. List these ideas on the board.

Example answers from a good 12th grade class:

Alter the pH, temperature, enzyme concentration (Note: this does not affect normal function but you might decide to allow the students to continue their experiment despite this since the important aspect is that the student is generating ideas and testing them.) At this point, you can also explain to them that salt concentration, and the presence/absence of metal ions (metal chelators like EDTA can remove such ions) also affect enzyme function.

An interesting connection to the biotechnology field can be made here. Point out to the students that none of the answers shown above can be used to treat aberrant enzyme function in a human. (I.e. you can't change the temperature of your cells at will.) Instead, scientists design small molecule inhibitors or activators that can selectively target an enzyme. This is a large focus of pharmaceutical industry as many sickness and illnesses can involve protein malfunction.

Some example small molecules to that can be used to affect function in this experiment are guanidinium chloride (good, relatively safe choice) and an amylase inhibitor that the SEP resoure center calls "Carbograbbers" (white kidney bean extract called phaseolamin).

PLEASE NOTE: Having the students come up with ideas, even possibly absurd ones (one group wanted to shine UV light on their protein) is the *most important part* of this entire lesson. As they plan their experiments, guide them in using good experimental practiced like replicates, controls, etc.

DAY 3: STUDENT DESIGNED EXPERIMENTS

This day is pretty fun for the students and the instructors. Give them some last minute reminders (i.e. stop timing after 15 minutes, try and do all the trials at the same time, move quickly).

The student-designed experiment may not show the results the students predicted or an error may be made in the experimental steps. When this happens (ie: adding amylase before iodine) tell give them positive feedback (i.e. "Great job. Now what do you think will happen when we add the iodine after adding the amylase?). Use this experimental errors as a teaching opportunity for working through and restratizing around mistakes.

Wrap-up / Closure:

Be sure to leave enough time at the end to go over the results. We got great results, with some things going as predicted and others seeming totally unexplained. The latter is an opportunity to discuss further experiments and the future directions aspect of experimental design. To allow for equal student participation consider using the Think-Pair-Share model.

Extensions and Reflections

Similar Lessons on SEP Lessons:
Amylase - Exploring digestion and evolution through a molecular machine

NGSS Topics

High School Life Sciences:
HS. Structure and Function

NGSS Disciplinary Core Ideas

High School (9-12):
HS-LS1 From Molecules to Organisms: Structure and Processes

NGSS Science and Engineering Practices

NGSS Science and Engineering Practices:
Asking Questions and Defining Problems
Planning and Carrying Out Investigations
Analyzing and Interpreting Data
Using Mathematics and Computational Thinking
Constructing Explanations and Designing Solutions
Engaging in Argument from Evidence

NGSS Crosscutting Concepts

NGSS Crosscutting Concepts:
Structure and Function

Standards - Grades 9-12 Investigation and Experimentation

Investigation and Experimentation:
a. Select and use appropriate tools and technology (such as computer-linked probes, spreadsheets, and graphing calculators) to perform tests, collect data, analyze relationships, and display data.
b. Identify and communicate sources of unavoidable experimental error.
c. Identify possible reasons for inconsistent results, such as sources of error or uncontrolled conditions.
d. Formulate explanations by using logic and evidence.
f. Distinguish between hypothesis and theory as scientific terms.
g. Recognize the usefulness and limitations of models and theories as scientific representations of reality.
j. Recognize the issues of statistical variability and the need for controlled tests.
l. Analyze situations and solve problems that require combining and applying concepts from more than one area of science.

Links:

- http://www.nextgenscience.org/hsls-sfip-structure-function-information-processing
- http://www.nextgenscience.org/hsls1-molecules-organisms-structures-processes

- http://www.nap.edu/openbook.php?record_id=18290&page=385
- http://www.nap.edu/openbook.php?record_id=18290&page=389
- http://www.nap.edu/openbook.php?record_id=18290&page=391
- http://www.nap.edu/openbook.php?record_id=18290&page=393
- http://www.nap.edu/openbook.php?record_id=18290&page=395
- http://www.nap.edu/openbook.php?record_id=18290&page=397
- http://www.nap.edu/openbook.php?record_id=18290&page=421