One: Observations of living things are recorded in the lab and in the field ...

Rationale

Observations must be made before hypotheses can be formulated and formal experiments designed. Techniques that will be used in a formal experiment must be practiced. Researchers take time to familiarize themselves with the organisms and systems that they will be working with in the laboratory before formal experimentation can begin. Students will use a floating disc assay system throughout their experiments with enzymes to test for catalase activity under various conditions. Before they can use the assay effectively to get good data in a formal experiment, they must first become familiar with handling the materials and recording appropriate observations. So, at this point, students will be given time to explore with minimal direction from the teacher.

Preview

Students will be learning to use an assay system consisting of a filter paper disc saturated with a yeast suspension. The yeast cells produce the catalase enzyme, so the disc which was soaked in the yeast suspension contains catalase. The disc with the yeast suspension initially drops to the bottom of the assay well. Catalase breaks down hydrogen peroxide into water and oxygen. Oxygen bubbles that form from the action of the enzyme get stuck in the paper disc. When enough oxygen bubbles accumulate, the disc actually rises to the top of the liquid layer. The time it takes for a disc to rise to the top of the liquid is an indicator of the rate of the decomposition reaction and the activity of the enzyme. Students' explorations will be directed towards examining the time it takes the disc to rise as they change the concentration of hydrogen peroxide. The version of a floating disc assay that students will be using is a micro-assay. It requires very small amounts of reagents: water and hydrogen peroxide and Bakers' yeast suspension. The materials they will use include a dropper, forceps, filter paper discs punched out of a piece of filter paper with a hole punch, and a 24-well microplate to hold their reactions. Each well in the plate holds 2.5 ml of liquid, and one 24-well plate provides 24 containers for doing the assay.

What's an assay? An assay is a systematic way to find out how something works. You measure or observe a specific parameter, in this case, time.

Materials

The version of the floating disc assay that students will be using is a micro-assay. It requires very small amounts of reagents: water and hydrogen peroxide and Bakers' yeast suspension. The materials they will use include a dropper, forceps, filter paper discs punched out of a piece of filter paper with a hole punch, and a 24-well microplate to hold their reactions. Each well in the plate holds 3 ml of liquid. One 24-well plate provides 24 containers for doing the assay.

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About thirty minutes before class prepare the yeast suspension to be used by the students.

For each class you will need:

- ✓ slightly warm water (about 37C)
- ✓ vessel: flask/beaker/bottle.
- ✓ water
- ✓ granulated sugar (sucrose)
- ✓ spoons
- ✓ one package (or one teaspoon) of yeast

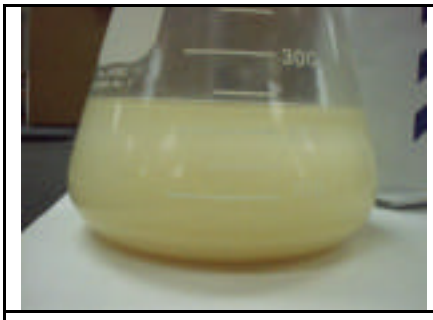
Baker's yeast can be obtained from the grocery store (use granulated form, avoid cakes which contain starch binder)

Make a fresh suspension for each class.

This will make about 10 ml of yeast suspension for each student:

- Add about 300 ml of warm water to a vessel (beaker, flask or bottle)
- Add 1 level teaspoon (1 packet) of yeast granules
- Swirl vigorously to mix
- Add 1 level teaspoon of sugar (sucrose)
- Swirl vigorously to stir
- Proof the yeast by letting it stand (incubate) for 30 minutes with occasional stirring/swirling

Though yeast are microbes, they settle fairly rapidly. If you bake yeast bread, you may have noticed the clump of yeast at the bottom of the proofing bowl. Dough that is not kneaded well will rise poorly because the yeast are not uniformly distributed through the mixture. For this experimental pathway, the enzyme source (yeast) also needs to be distributed well, or students will note large errors between test sets as well as within an assay among duplicate wells.

		
Fresh suspension	Settling after 15 minutes (note the thin clear layer)	After 30 minutes, note clearing to below the 200 ml line