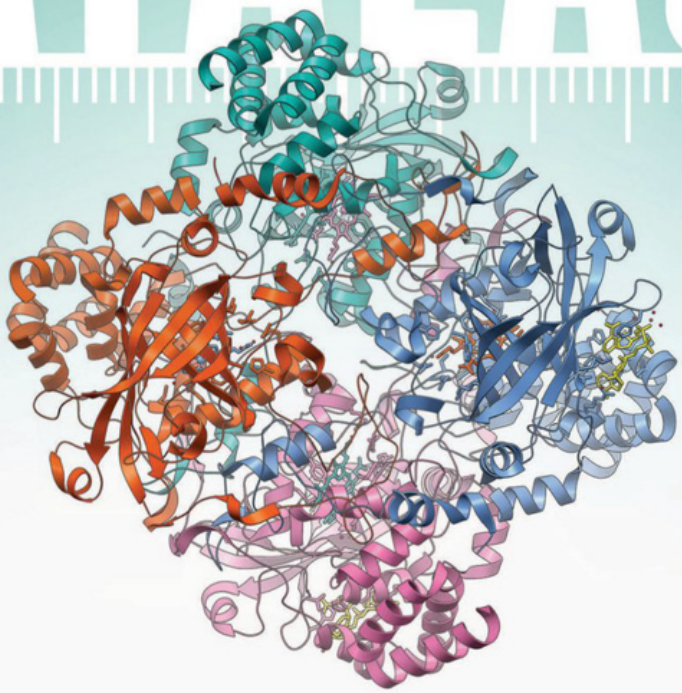
A TWIST ON MEASURING CATALASE



A more consistent—and fun—activity that explores enzyme reactions

Pamela Bryer

Catalase, an enzyme found in both plant and animal cells, prevents the accumulation of toxic levels of hydrogen peroxide (H_2O_2) by catalyzing its decomposition to water and oxygen gas. Because this enzyme is ubiquitous, it is frequently used in high school biology laboratories to explore enzyme reactions.

One common method for measuring catalase activity is to dip paper disks in a yeast or potato solution (the source of catalase) and place the disks in a container of hydrogen peroxide (see "On the web"). The disks initially sink, but as catalase reacts with the H_2O_2, oxygen (O_2) is produced, and the disks rise because oxygen bubbles attach to the disks.

Above: Catalase protein structure rendering. VOSSMAN

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

FIGURE 1

Dropping yeast–sodium alginate solution into $CaCl_2$ to form spheres.

ALL PHOTOS COURTESY OF THE AUTHOR

FIGURE 2

Yeast–sodium alginate spheres.

Although this is an easy experiment to perform, some variables are difficult to control (e.g., the amount of yeast or potato solution on the disk). This article explores an equally easy and perhaps more enjoyable way to do the same experiment while reducing the inherent variability of the paper disk design. Instead of paper disks, students use yeast–sodium alginate spheres to get reliable and consistent results.

To form uniform spheres, yeast cells are encapsulated in *sodium alginate*, a nontoxic algal extract. These spheres are dropped into H_2O_2, and the students measure the time it takes for the spheres to rise. The potential for inquiry-based experiments abound: What happens if the temperature is changed or the pH or H_2O_2 concentration? Because the reaction is fairly quick, multiple replicates can be performed in a short amount of time. Therefore, statistics may be used to interpret the data.

Preparing the yeast–sodium alginate solution

1. Add equal volumes of a 10% yeast solution to a 2% sodium alginate solution. Mix well with a glass rod.
2. Draw up the yeast–sodium alginate solution into a 30 ml plastic syringe (without needle). Carefully wipe off all excess liquid from the syringe tip.

Making the yeast spheres

1. Hold the syringe containing the yeast–sodium alginate solution over a beaker or cup filled 1/3–1/2 full with 0.15 M $CaCl_2$ (calcium chloride). Depress the plunger very slowly so that a drop of the yeast–sodium alginate solution falls into the beaker. A sphere should form as the drop comes in contact with the $CaCl_2$ solution and falls to the bottom of the beaker (Figure 1).
2. Continue releasing yeast–sodium alginate drops into the 0.15 M $CaCl_2$ solution. Try to use even pressure so spheres are of uniform size.
3. The spheres should remain in this solution for about five minutes to harden.
4. Dispose of any floating spheres.
5. Obtain a small strainer and hold it over a clean beaker or cup. Very carefully pour the contents of the beaker/cup containing the yeast–sodium alginate spheres into the strainer (Figure 2).
6. Once drained, carefully rinse the spheres under a slow-running faucet or with tap water from a wash bottle.

7. Pour the spheres into a cup or beaker. If not using the spheres immediately, add water so they don't dry out.

Testing the catalase reaction

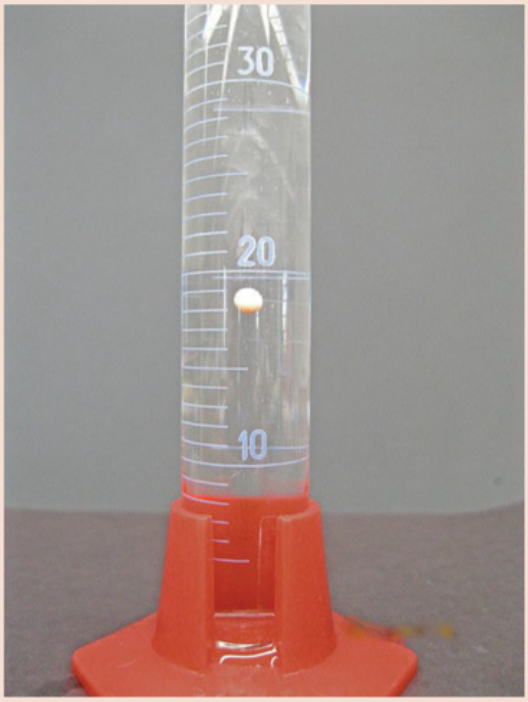
1. Pour 50 ml of a 0.3% H_2O_2 solution into a 50 ml graduated cylinder. You will need to dilute commercial H_2O_2, which typically has a concentration of 3%.
2. Using forceps, or the loop end of the inoculating loop, gently remove one yeast sphere from the cup or beaker.
3. Drop the sphere into the graduated cylinder. Decide when to start timing: as soon as the sphere touches the surface of the hydrogen peroxide or as soon as it touches the bottom of the cylinder. Keep timing until the sphere reaches the surface again (Figure 3).
4. Dispose of the yeast sphere.
5. Do this with a few more spheres to get the timing down.

Designing an experiment

1. Decide what variable to test. Possibilities include substrate concentration (H_2O_2), temperature, or pH.
2. Write out your experimental design. Things to think about: What is the control? What concentration(s) of substrate will be used? What temperature(s)? How many trials? How will data be displayed?
3. The unused yeast spheres may be kept and used the following day if they are refrigerated.

FIGURE 3

Yeast–sodium alginate sphere rises in H_2O_2.



Notes on the activity

Supplies and preparation

- Sodium alginate is used as a thickening agent in foods, such as ice cream, yogurt, and cake mixes, because it helps to emulsify oil and water and gives a smooth texture to foods. It may be purchased from Flinn Scientific, Amazon, or gourmet supply stores (some chefs use it to make flavor "pearls" or "caviar," and it is also used to encapsulate yeast in the production of wine). I have found no difference in either stability of spheres or activity between spheres made with the different sodium alginate powders.
- I make a 2% sodium alginate solution (for 20 ml, use 0.4g/20ml of distilled water, or dH_2O) a day or two before it is to be used because it takes a long time to turn sodium alginate into a solution. I just weigh out the amount I need into a beaker or plastic cup, add dH_2O (preferable to tap water), stir, and leave it out at room temperature. It may be put in the refrigerator, too, but it is fine for days at room temperature. Because the solution is so viscous I would recommend making a separate beaker/cup for each group instead of making a big batch and trying to portion it out the next day.
- I use Fleischman's RapidRise bread yeast to make a 10% yeast solution in water (10g yeast/100 ml warm water; tap water is fine). Make this solution about 5–10 minutes before use.
- Hydrogen peroxide may be found in the health and beauty section at most grocery or department stores or any drugstore. It comes as a 3% solution. Use a new container of hydrogen peroxide because it can lose its effectiveness over time.
- $CaCl_2$ is available from any science supply company. I use calcium chloride dihydrate. Make enough 0.15 M $CaCl_2$ (11g $CaCl_2 \cdot 2H_2O$/500 ml dH_2O) so each group can have 50 ml.

Yeast sphere troubleshooting

- When sodium alginate, a hydrophilic polymer, comes in contact with $CaCl_2$, sodium ions are replaced with

FIGURE 4

The effect of temperature on the rise of yeast-sodium alginate spheres.

The time for yeast–sodium alginate spheres to rise to the top of cylinders containing different temperatures of H_2O_2 was measured. As the temperature increased, the time to rise to the surface decreased (ANOVA, $p<0.0001$).

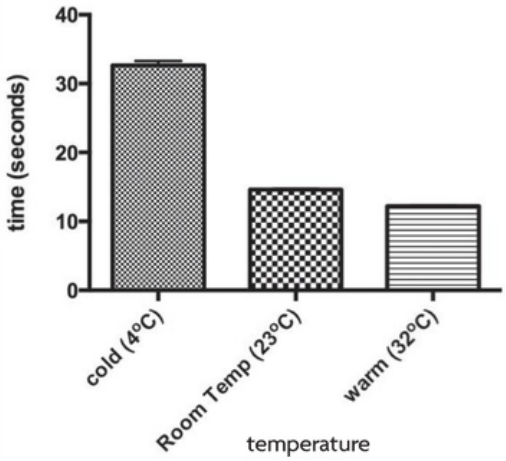
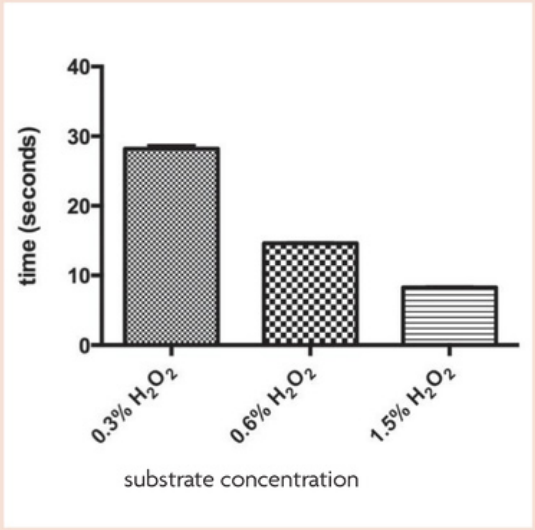


FIGURE 5

The effect of substrate concentration.

As the substrate concentration (H_2O_2) increased, the yeast–sodium alginate spheres more quickly reached the surface (ANOVA, $p<0.0001$).



calcium. This leads to cross-linkages between the polymer chains, and an insoluble gel is formed. If spheres with "tails" form, the yeast–sodium alginate solution may be too thick; add a dash of dH_2O to thin it out.

- It is important for students to use spheres that are close in size for their trials. Yeast spheres of different sizes are usually due to an uneven pressure on the syringe plunger when dropping the solution into $CaCl_2$.
- Discard any floating spheres. These are usually due to an uneven suspension of the yeast–sodium alginate solution.
- Once the yeast spheres are made, they can be stored either in a Ziploc bag without water or in a beaker or plastic bag with tap water or dH_2O in the refrigerator. Activity did not diminish no matter how they were stored. I left some out overnight in all three conditions, and they were fine. Just don't let them dry out.

Setting up experiments

- For my runs of different substrate concentrations (Figures 5 and 7), I used 0.9% H_2O_2 (15 ml H_2O_2 + 35 ml H_2O); 0.6% H_2O_2 (10 ml H_2O_2 + 40 ml H_2O); 0.3% H_2O_2 (5 ml H_2O_2 + 45 ml H_2O); and 0.12% (2 ml H_2O_2 + 48 ml H_2O). Students can start with the 0.3% they used for their initial trial and decide what concentrations to use based on the timing of that trial.
- For experiments that investigate enzyme activity at different temperatures, make sure the H_2O_2 and the yeast spheres are at the right temperature before the experiment proceeds. Make sure students work quickly so the temperature of the spheres or the H_2O_2 doesn't get back to room temperature before they are finished. Before conducting the experiment, the spheres may be put in water at the desired temperature to equilibrate.
- To test the effect of pH on the reaction rate, I soaked the spheres for 5–10 minutes in the pH buffer and made the H_2O_2 dilutions in the pH buffer. At high and low pH, the spheres may start to disintegrate if left in the buffer to equilibrate for too long.

FIGURE 6

The effect of temperature on the rate in which catalase reacts to H_2O_2.

The effect of temperature was measured on the rate of the conversion of hydrogen peroxide to water and oxygen gas by the enzyme catalase by the movement of yeast–sodium alginate spheres. As temperature increased, the rate of reaction increased (ANOVA, p<0.0001).

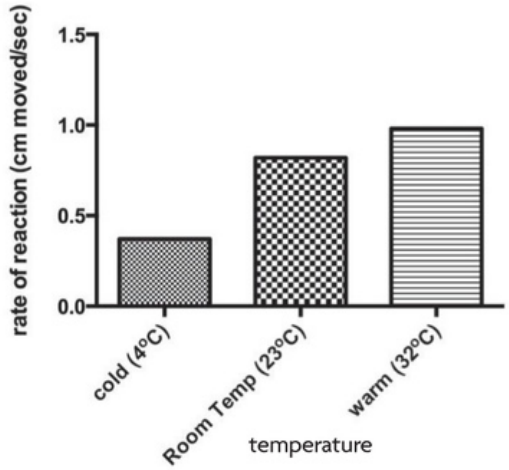
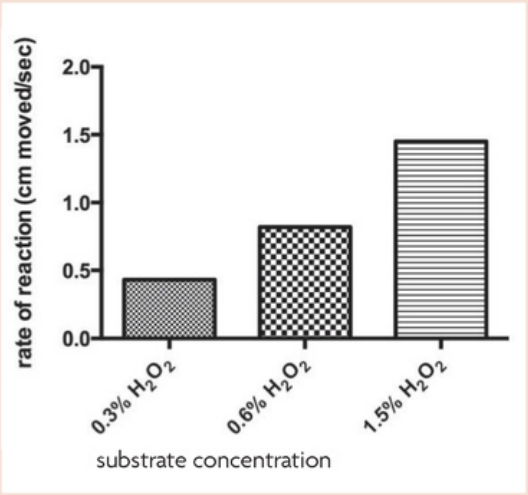


FIGURE 7

The effect of substrate concentration (H_2O_2) on the catalase reaction.

Rate of catalase reaction was measured as a function of substrate concentration. As substrate concentration (H_2O_2) increased, the rate of reaction increased (ANOVA, p<0.0001).



Data analysis

- Students may graph data by the time it takes the yeast spheres to rise to the surface (Figures 4 and 5), or they may convert to rate of reaction by dividing the distance the spheres rose by the time (Figures 6 and 7).
- Statistical analysis (ANOVA or t-Tests) showed there was a significant difference in the reaction rate for temperature changes, different substrate concentrations, and pH.

Safety precautions

- Because yeast and sodium alginate are considered to be nonhazardous, yeast spheres can safely be disposed of in the trash.
- Calcium chloride is considered to be a mild body tissue irritant and slightly toxic by ingestion. Excess calcium chloride solution may be safely disposed of according to local regulations.

Conclusion

With yeast spheres, there is no issue with variable amounts of enzyme from trial to trial. It's easy to manipulate the yeast spheres, unlike the paper disks that are difficult to pick up and often stick to the side of containers. Because large numbers of yeast spheres may be made at once and retain their viability over several days, investigations can be done with the same batch of yeast spheres for a day or two. This enables students to experiment, design procedures, and carry out multiple trials for each condition with the same batch of spheres. It also gives students a large data set to use for statistical analysis. I have used this procedure with high school students, college students, and teachers in workshops, always with positive results. Everyone enjoys making the spheres and doing the experiments. ■

Pamela Bryer (pbryer@bowdoin.edu) *is director of laboratories and a lab instructor at Bowdoin College in Brunswick, Maine.*

On the web

Traditional catalase experiment: *http://cibt.cornell.edu/labs-activities/labs/catalase/*