

A Twist on Measuring Catalase Activity – Using Sodium Alginate-Yeast Spheres
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Catalase, found in both plant and animal cells, has the extremely important function of preventing the accumulation of toxic levels of the strong oxidizing agent hydrogen peroxide (H_2O_2), a by-product of metabolic processes. Because this enzyme is so ubiquitous it is frequently used in high school biology laboratories to explore enzyme reactions. One common method is to dip paper disks in a yeast or potato solution (http://cibt.bio.cornell.edu/labs_and_activities/index.php) and place the disks in a container of hydrogen peroxide. The disks initially sink but as catalase reacts with the H_2O_2 , O_2 is produced and the disks rise.



While this is an easy experiment to perform there are some variables that are difficult to control (e.g. the amount of yeast or potato solution on the disk). This workshop will explore an equally easy and perhaps more enjoyable way to do the same experiment that reduces the inherent variability of the paper disk design. Yeast cells are encapsulated in sodium alginate, a non-toxic, easily obtainable, algal extract, to form uniform spheres. These spheres are dropped into H_2O_2 and the time for the spheres to rise is measured. The potential for inquiry-based experiments abound; what happens if the temperature is changed, pH, H_2O_2 concentration? Because the reaction is fairly quick, multiple replicates can be performed in a short amount of time so statistics may be used to interpret the data.

General procedure:

(Directions for this workshop are in italics in parentheses for each step)

Preparing the yeast/sodium alginate solution

1. Add equal volumes of a 10% yeast (*Saccharomyces cerevisiae*) solution to a 2% sodium alginate solution (this solution is very viscous). Mix well with a glass rod. *(20 ml of each, mix with end of inoculating loop)*
2. Draw up the yeast -sodium alginate solution into a 30 ml syringe. 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 containing 50 ml of 0.15M CaCl_2 . *(hold over plastic cup about 1/3 full with 0.15M CaCl_2)*. Very slowly depress the plunger 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 fall to the bottom of the beaker.
2. Continue releasing yeast-sodium alginate drops into the 0.15M CaCl_2 solution. Try to have spheres all of uniform size. The spheres should remain in this solution for about 5 minutes to harden.
3. Dispose of any floating spheres.
4. Obtain a small strainer and hold it over a clean beaker *(hold over clean cup)*. Very carefully pour the contents of the beaker containing the yeast-sodium alginate spheres into the strainer.
5. Once drained, carefully rinse the spheres under a slow running faucet *(rinse spheres with tap water from squirt bottle)*.
6. Pour the spheres into a petri dish or into a beaker *(just take spheres out of strainer as needed)*. If not using immediately add 25 ml of water so the spheres don't dry out.

Testing the catalase reaction

1. Pour 50 ml of a 0.6% hydrogen peroxide (H_2O_2) solution into a 50 ml graduated cylinder.
2. Using forceps, *(use the loop end of the inoculating loop)* gently remove one yeast sphere from the petri dish or beaker *(strainer)*.
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.
4. Dispose of the yeast sphere.
5. Do this with a few more spheres to get the timing down.

Designing an experiment

Once the basics of this set-up are understood, students are free to design an experiment to see what effect some variable will have on this enzymatic reaction. Make sure they write out their experimental design first before carrying out the experiment. What is the control? What concentration(s) of substrate will be used? What temperature(s)? How many trials? The unused yeast spheres may be kept and used the following day as long as they are refrigerated.

Materials and notes

1. Sodium alginate is a non-toxic hydrophilic polysaccharide that 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 give a smooth texture to foods. It may be purchased from Flinn (S0445, 25g/\$13), online from Amazon (400g/\$25), or it may be found in gourmet supply stores (it is used by some chefs to make flavor “pearls” or “caviar” and it is also used to encapsulate yeast in the production of wine).
I make a 2% sodium alginate solution the day before it is to be used because it takes a long time to get into solution. I just weigh out the amount I need into a beaker, add dH₂O (not tap), stir, and put it into the fridge overnight (I’ve also inadvertently left it out overnight and it is fine). Because the solution is so viscous I would recommend making this up in beakers, and making a separate beaker for each group instead of making a big batch and trying to aliquot it out the next day.
2. I use Fleischman’s RapidRise bread yeast to make a 10% yeast solution in dH₂O (not tap). Make this up about 10 minutes before it will be used.
3. 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. Be sure to use fresh as containers opened for long periods of time can lose their effectiveness.
4. CaCl₂ is available from any science supply company. Make up enough 0.15M CaCl₂ (11g/500 ml dH₂O) so each group will have 50 ml.
5. For my trial runs of different substrate concentrations I used 1.5% H₂O₂ (25 ml H₂O₂ + 25 ml dH₂O), 0.6% H₂O₂ (10 ml H₂O₂ + 40 ml dH₂O), and 0.3% H₂O₂ (5 ml H₂O₂ + 45 ml dH₂O).
6. For experiments that investigate different temperatures make sure the H₂O₂ and the yeast spheres are to the temperature to be tested before the experiment proceeds. Make sure students work quickly when looking at different temperatures so the temperature of the spheres or the H₂O₂ doesn’t get back to room temperature before they are done. The spheres may be put in water at the desired temperature to equilibrate.
7. Once the yeast spheres are made they can be stored either in a plastic baggie without water, or in a beaker with either tap water or dH₂O in the refrigerator. I’ve stored them all three ways to see if activity diminishes and it doesn’t for several days no matter what way they were stored. I even left some out overnight in all three conditions and they were fine. Just be sure they don’t dry out.
8. When sodium alginate, a hydrophilic polymer, comes in contact with CaCl₂, sodium ions are replaced with calcium. This leads to cross-linkages between the polymer chains and an insoluble gel is formed. If spheres with “tails” form when the yeast-sodium alginate solution comes in contact with the CaCl₂ the yeast-sodium alginate solution may be too thick. If this is the case just thin it out with a bit of dH₂O.
9. Students may graph data either by the time it takes the yeast spheres to rise to surface or they may convert to rate of reaction by dividing the distance the spheres rose by the time
10. Statistical analysis (ANOVA) showed there was a significant difference in the reaction rate for both temperature changes (rate increased as temperature increased) and substrate concentration (rate increased as the substrate concentration increased)
11. I have also used the yeast spheres to test the effect of pH on the reaction rate. I soaked the spheres for 10 minutes in the pH buffer to be tested and made the H₂O₂ dilutions in the pH buffer. At high and low pH the spheres can start to disintegrate.
12. Safety precautions – both yeast and sodium alginate are considered to be non-hazardous so the 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 rinsed down the drain with plenty of water.

I have used this procedure with high school students, college students and introduced it to teachers in workshops with always positive results and comments. Everyone enjoys making the spheres and then doing the experiment. I have since incorporated use of the spheres into respiration experiments with equally positive results.