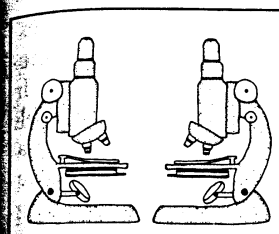

How To Do It

pH & Rate of Enzymatic Reactions

Roy B. Clariana

This article describes a quantitative and very inexpensive way to measure the rate of an enzymatic reaction. The laboratory activity deals with the effects of different pH levels on the rate of reaction; however, the methodology can be easily adapted for measuring the effects of temperature, substrate concentration, enzyme concentration and reaction inhibitors. Also, the approach can be used either as a demonstration or as a student laboratory activity.

Molecules of hydrogen peroxide are broken down in the presence of the enzyme catalase. A yeast suspension is used to provide the catalase, although we have also used crushed potatoes with the same results. Small filter paper squares are dipped into a yeast suspension and then dropped in a very dilute hydrogen peroxide solution. The filter paper square sinks to the bottom due to its density (note: filter paper is used because ordinary paper will not easily sink when dropped in water). The catalase on the filter paper begins to react with the hydrogen peroxide, producing small oxygen bubbles. These bubbles are trapped in the fibers of the filter paper square. Eventually the paper will float to the surface (Figure 1). The time required for this to occur depends directly on the rate of the reaction. Obviously, the faster the reaction, the faster the paper will float. The optimum pH for catalase function is 6.8. The further the pH is from this optimum value, the slower the reaction rate. Thus in this activity, a graph of the rate of reaction versus pH will produce a normal curve with an optimum at pH 7.

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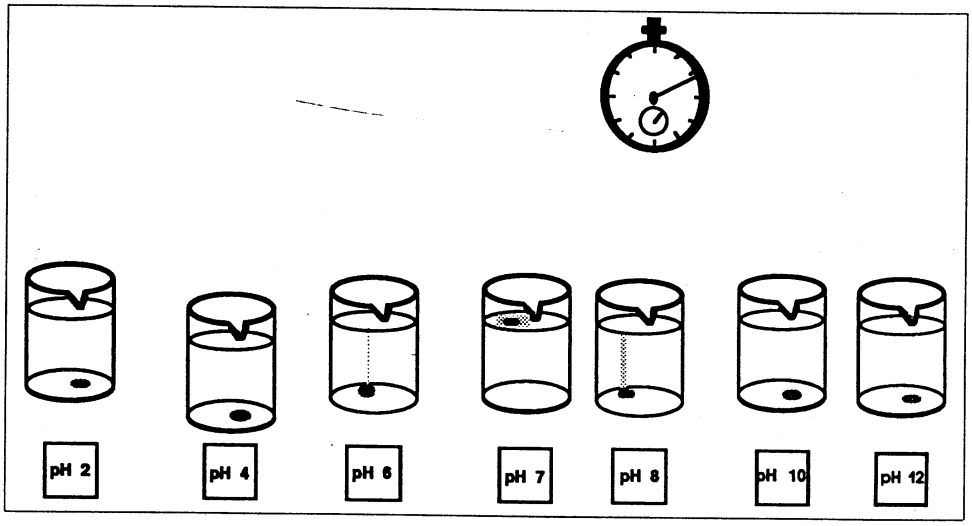

Figure 1. These beakers contain dilute hydrogen peroxide solutions. The enzyme on the square pieces of filter paper in each cylinder causes the production of oxygen which then causes the pieces to float. The rate of enzymatic reaction was highest at pH 7, as indicated by the floating piece of paper in that cylinder.

Materials

Hydrogen peroxide
Packet of yeast
Scissors (or razor blade)
Ruler (mm)
Stopwatch
Several small forceps
Filter paper (e.g. number 3)
7 beakers for each group of students (apx. 100 ml to 250 ml)
1 graduated cylinder (apx. 500 ml) and several containers for preparing solutions
Paper labels and pen
Hydrochloric acid and sodium hydroxide

Preparation

Prepare the following materials beforehand (or have the students prepare the following):

1. Mix the yeast in a beaker containing warm water. Exact measurements of water and yeast are not critical. Allow about 30 minutes for the yeast to activate. Label this mixture "catalase" or "enzyme."
2. Cut the number 3 filter paper circles into squares that are 5 mm on a side. Try to avoid excessive contact with the paper and be sure your hands are as clean as possible. Oils from your hands can alter the paper's ability to absorb the catalase solution.
3. Using water (tap water is OK), prepare a 1:1000 dilution of the hydrogen peroxide in the 500 ml cylinder. Inexpensive and easily obtained hydrogen peroxide from a supermarket or drug store works fine. Depending upon the freshness of this solution, additional dilution may be required. To test this solution, pour about 50 ml of the diluted hydrogen peroxide into a beaker. Use the forceps to dip a filter paper square into the catalase (i.e. yeast) mixture and drop the filter paper square into the beaker. The paper should sink completely to the bottom and then float to the top in about 40 seconds. If it takes less time, add water to further dilute the peroxide; if it takes too much time, add more hydrogen peroxide, and test again. You