How-To-Do-It

Apparatus & Experimental Design for Measuring Fermentation Rates in Yeast

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Fermentation is an aerobic process by which certain organisms extract energy from sugars. As sugars are fermented, adenosine triphosphate (ATP) is synthesized while carbon dioxide and ethanol are produced. Except for the last two steps of fermentation, in which carbon dioxide and ethanol are produced, fermentation is the same as glycolysis.

Yeast has been an ideal organism in which to study fermentation ever since the pioneering works of Louis Pasteur, who connected the process to living organisms, and Eduard Buchner, who discovered that cell-free extracts of yeast ferment glucose to ethanol (Wolfe 1981). Since then, yeast has often been used in biology classes to demonstrate fermentation.

The reactions of fermentation are catalyzed by about 12 different enzymes working in tandem. Several of the enzymes have been studied in detail and shown to be affected by metabolic poisons and other compounds.

1) Phosphofructokinase, the allosteric enzyme which adds a phosphate ion from ATP to fructose-6-phosphate forming frucose-1,6-diphosphate, is inhibited by citrate ions (Lehninger 1975).
2) Aldolase, which catalyzes the hydrolysis of fructose-1,6-diphosphate into dihydroxyacetone-3-phosphate and glyceraldehyde-3-phosphate, is inactivated by the amino acid cysteine. This inactivation may be reversed by zinc, ferrous or cobalt ions (Conn & Stumpf 1966).
3) Glyceraldehyde dehydrogenase, which oxidizes and phosphorylates glyceraldehyde-3-phosphate, is inhibited by mercuric and iodacetate ions, probably by interference at the active site on the enzyme (Lehninger 1975).
4) Enolase, which dehydrates 2-phosphoglyceric acid to form 2-phosphoenolpyruvic acid, is inhibited by fluoride ions, presumably by complexing with magnesium or manganese cofactors required to activate the enzyme (Lehninger 1975).

In adddition to these enzymes, yeast also contains invertase (=sucrase) which hydrolyzes sucrose into glucose and fructose, enabling yeast cells to utilize sucrose in fermentation.

In this article I describe an apparatus that facilitates the quantitative study of fermentation in yeast by allowing simultaneous measurements of fermentation rates in several treatments and a control. In addition, I describe a laboratory procedure in which the apparatus is used and present several suggestions for further investigations.

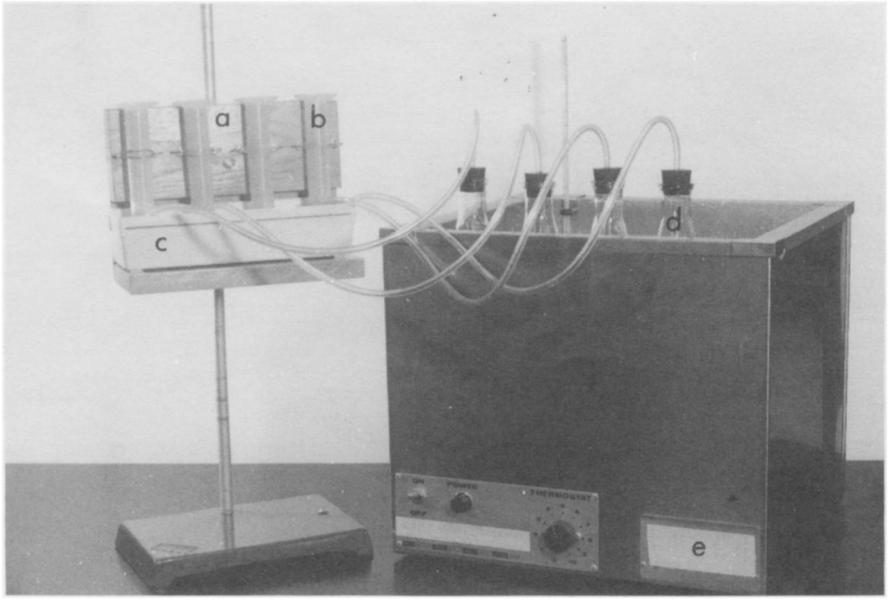

Figure 1. Apparatus set up for measuring fermentation rates. (a) Wooden support with cup hooks. (b) 25 ml graduated cylinder held in place by rubber band attached to cup hooks. (c) Pneumatic trough made of a 9-inch × 3-inch × 2-inch drawer organizer. (d) 125 ml Erlenmeyer flask. (e) Hot water bath.

Constructing the Apparatus

Figure 1 shows the complete apparatus set up for measuring fermentation rates. Of the items shown, the only ones that require construction and assembly are the graduated cylinder-pneumatic trough support and the tubing that connects the fermentation flasks to the graduated cylinders. All materials are available at local lumber, hardware and department stores or from biological supply houses. Construction time and costs are minimal.

The support is constructed from 4-inch by 1-inch lumber and ⅜-inch plywood. Exact dimensions are given in Figure 2. The three pieces of wood are nailed and glued together as shown. Eight ⅝-inch brass cup hooks, spaced

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as shown in Figure 2, form the attachment points for rubber bands which hold four graduated cylinders in place. The ringstand mounting (Figure 3) is made from the ringstand clamp end of a buret clamp. The clamp is fastened to the back of the support with a 1½-inch by ¼-inch round head machine screw (Figure 4). A washer, installed between the wooden support and the clamp, prevents the clamp, when tightened, from gouging the wood. Before the hardware is added, the wooden support should be painted with enamel or varnish to prevent water damage and warping.

The hose that connects each fermentation flask to a graduated cylinder is a two-foot pice of ¼-inch i.d. Tygon tubing. One end of the tubing is inserted directly into a #4 or #5 one-hole rubber stopper (Figure 3). The other end is fitted with a 3/16-inch o.d. piece of glass tubing which has been bent so that a 30-degree angle separates one leg, which is one inch

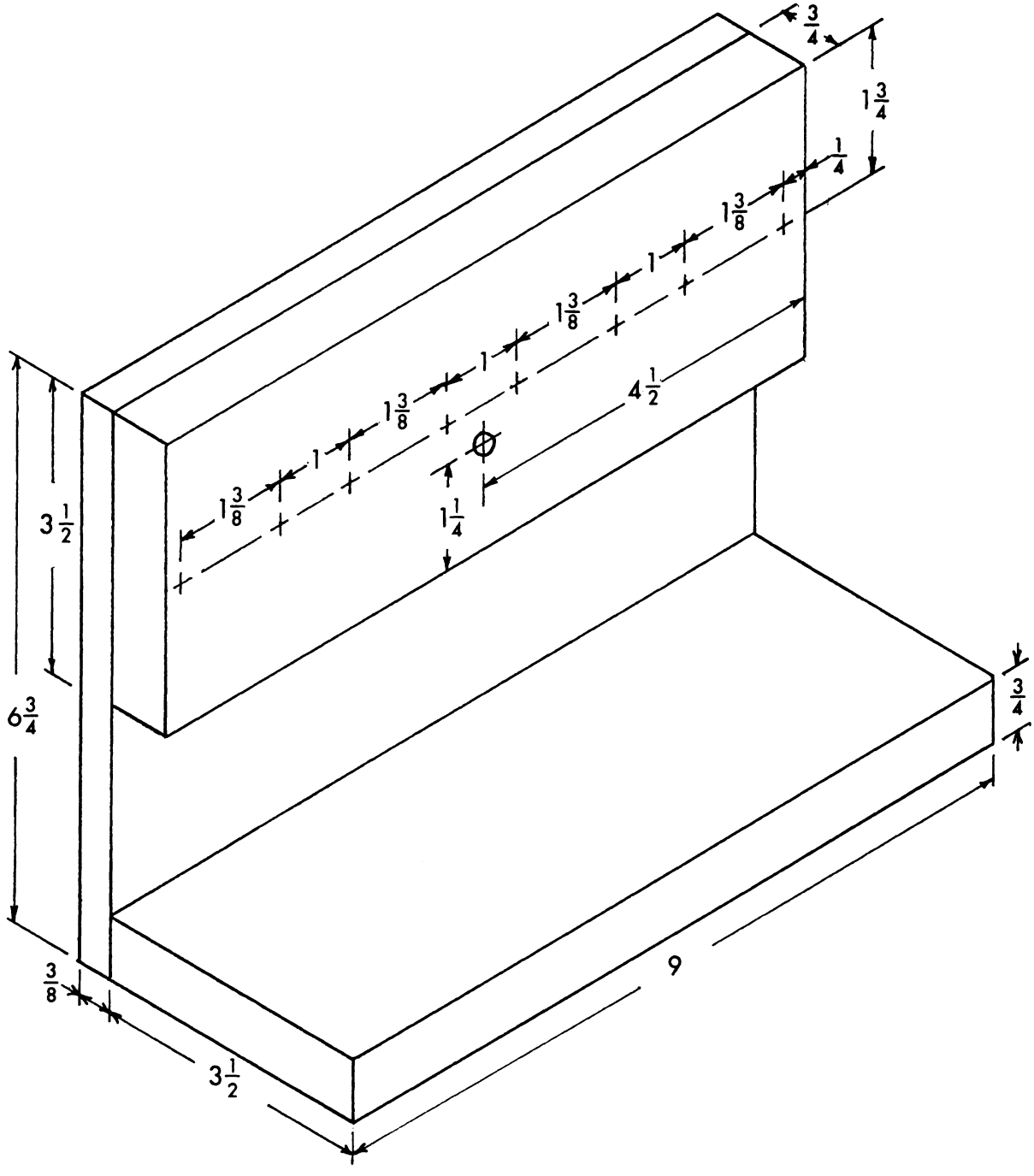

Figure 2. Construction details for pneumatic trough-gas collection support. All dimensions are given in inches. The hole in the vertical piece is ¼ inch in diameter and accommodates a 1½ inch × ¼ inch round head machine screw. The line of centers above the hole are locations for the cup hooks.

long, from the other, which is about two inches long. (When inserted up into a graduated cylinder, the long leg should not extend beyond the first measuring mark on the graduated cylinder.)

Additional equipment include a pneumatic trough (a 9-inch by 3-inch by 2-inch drawer organizer works well), four small rubber bands and four 25 ml graduated cylinders.

Conducting an Investigation

This investigation directs students to measure the rates of fermentation in yeast by trapping and determining the volume of carbon dioxide as it displaces water contained in inverted graduated cylinders. In the process they will examine the effects of several respiratory poisons on these fermentation rates. The laboratory exercise that follows has been student-tested so that it works without pitfalls. The total time for completion is about 100 minutes, but that can be shortened if the yeast suspensions have been prepared in advance and if the running time of the experiment is reduced.

1) Prepare the yeast fermentation flasks by marking the necks of four 125 ml Erlenmeyer flasks to indicate the four treatments—Control, Mercury, Cysteine and Fluoride.
2) To a 500 ml Erlenmeyer flask add 400 ml of distilled water and 20 g of sucrose. Swirl the flasks until the sucrose is dissolved.
3) Make the following additions to the appropriate flask:
   a. Control—95 ml of sucrose solution and 5 ml of distilled water.
   b. Mercury—95 ml of sucrose solution and 5 ml of 1 percent (w/v) mercuric chloride.
   c. Cysteine—95 ml of sucrose solution and 5 ml of 1 percent (w/v) cysteine.
   d. Fluoride—95 ml of sucrose solution and 5 ml of 1 percent (w/v) sodium fluoride.
4) To each flask add 0.5 g of active dry yeast and mix thoroughly by swirling.
5) Incubate the flasks for 10 minutes in a water bath at 38–40 degrees to bring their contents to the temperature of the water in the water bath.
6) While the flasks are incubating, attach the wooden support to a ringstand so the bottom of the support is even with the top of the water bath. Then fill a plastic pneumatic trough with water and place it on the shelf of the wooden support.
7) In succession, fill four 25 ml graduated cylinders with water by immersing them in the trough. Then invert each graduated cylinder without removing the open end from the water in the trough. Affix each graduated cylinder to the wooden support with a rubber band connnected between adjacent hooks.
8) After the 10-minute incubation period, seal each Erlenmeyer flask with a moistened one-hole rubber stopper which has been fitted with flexible tubing. Then insert the glass tubing end into a graduated cylinder without allowing the water to drain from the cylinder.
9) Begin timing the experiment. In Table 1 record the initial volume of gas in each graduated cylinder. Then, at 10-minute intervals for one hour, read and record the volume of gas in each cylinder.

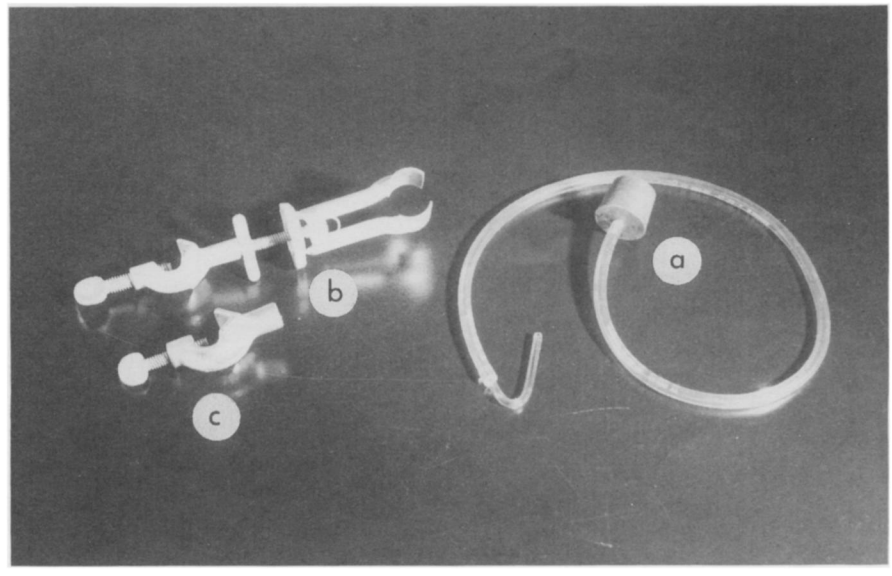

Figure 3. (a) Rubber stopper and tubing used to connect the fermentation flasks to the graduated cylinders. The glass tubing, inserted into the Tygon tubing, has a 30-degree bend. Buret clamp (b) and the ringstand clamp end (c) of a buret are shown at left.

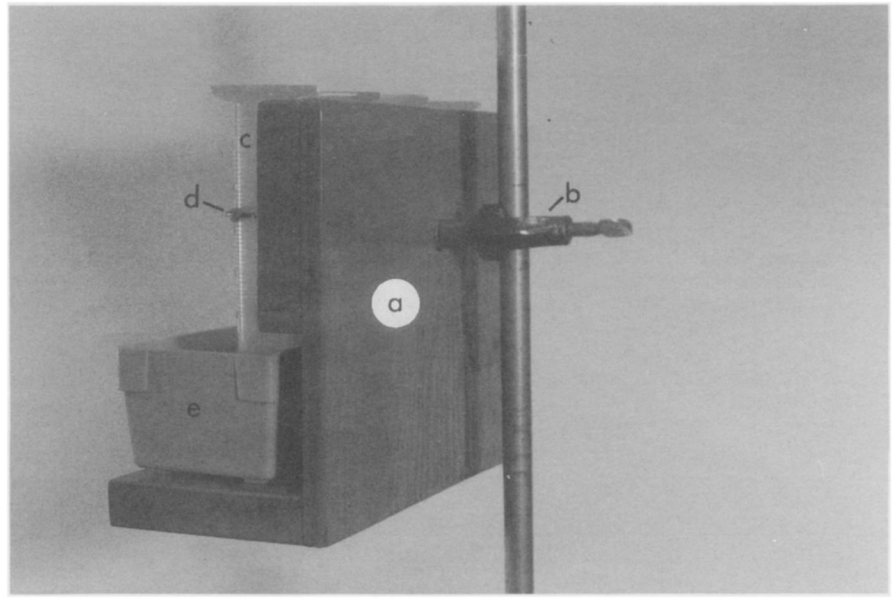

Figure 4. Back and side view of wooden support (a) showing the attachment of the buret clamp (b), the graduated cylinders (c), each held in place by a rubber band attached to a pair of cup hooks (d), and the pneumatic trough (e).

10) After you have completed the experiment, empty the contents of each flask down the drain. Run additional water from the tap to flush the drain of liquids.

Evaluation & Extension

Table 2 shows statistics typical for the investigation described above. Each of the substances significantly depressed the rate of carbon dioxide production when compared to the control. These reductions in carbon dioxide production would be expected because each of the substances in-

Table 1. Volumes of carbon dioxide produced by yeast treated with metabolic inhibitors. Volumes are in ml.

Time	Treatments			
	Control	Mercury	Cysteine	Fluoride
0				
10				
20				
30				
40				
50				
60				

hibits one of the enzymes participating in glycolysis.

By slightly modifying the procedure, other research questions may be answered. For example, can yeast ferment sugars other than sucrose? In general, the answer is yes, if the sugar can permeate the yeast cell membrane and if the yeast contains enzymes that will allow the sugar to enter the glycolytic pathway.

I have tried 3 percent solutions of the monosaccharides glucose, fructose, galactose, mannose and xylose and 3 percent solutions of the disaccharides maltose, lactose and cellibiose. Of these, only glucose, fructose and mannose were fermented (Table 3). Galactose and xylose were not fermented because yeast hexokinase (the entry enzyme of glycolysis) will not phosphorylate them (Eddy 1958). Lactose and cellibiose were not fermented because the yeast cell membrane is not permeable to them (Eddy 1958). Although some strains of yeast can ferment maltose (Nord & Weiss 1958), the strain found in Fleishmann's active dry yeast cannot, probably because it lacks a maltose hydrolyzing enzyme.

The apparatus and procedures I have described are very versatile and will allow many other variables to be examined. These include temperature, pH, ethanol concentration and different brands of commercially available yeast. I am sure your students can think of many more.

Table 3. Fermentation rates of Fleishmann's active dry yeast in various sugar solutions. Each treatment contained 0.5 g of yeast in 100 ml of 3 percent (w/v) sugar solution. The experiments were run at 40 degrees Celsius. Values are means and standard deviations for three replicates.

Treatment	*ml CO_2/h*
Monosaccharides	
Glucose	19.0 + 1.5
Fructose	19.0 + 0.5
Mannose	18.2 + 1.4
Galactose	1.3 + 0.8
Xylose	1.2 + 0.3
Disaccharides	
Sucrose	20.8 + 2.3
Maltose	1.0 + 0.0
Lactose	0.7 + 0.3
Cellibiose	0.0 + 0.0

Table 2. Mean volumes (ml) of carbon dioxide produced by yeast at 38-40 degrees Celsius. Each treatment contained 95 ml of 5 percent sucrose, 0.5 g of yeast and 5 ml of distilled water, 1 percent mercuric chloride, 1 percent cysteine or 1 percent sodium fluoride. Except for the initial readings (time = 0), all other means are significantly different from the control means as judged by a Student's t test (N = 6, d.f. = 10). * = $p < 0.01$ ** = $p < 0.001$

	Treatments			
Time (minutes)	*Control*	*Mercury*	*Cysteine*	*Fluoride*
0	0.00	0.00	0.00	0.00
10	4.50	0.25**	1.92*	0.58**
20	8.67	0.42**	4.25*	2.08**
30	12.42	0.67**	6.08**	4.25**
40	15.00	0.67**	7.33**	5.25**
50	17.17	0.83**	8.58**	6.58**
60	18.92	0.92**	9.33**	7.67**

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