

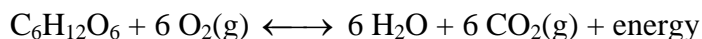
AEROBIC RESPIRATION

LAB DO 2.CALC

From *Biology with Calculators*, Vernier Software & Technology, 2000.

INTRODUCTION

Aerobic cellular respiration is the process of converting the chemical energy of organic molecules into a form immediately usable by organisms. Glucose may be oxidized completely if sufficient oxygen is available, by the following reaction:



All organisms, including plants and animals, oxidize glucose for energy. Often, this energy is used to convert ADP and phosphate into ATP. In this experiment, the rate of cellular respiration will be measured by monitoring the consumption of oxygen gas.

Many environmental variables might affect the rate of aerobic cellular respiration. Temperature changes have profound effects upon living things. Enzyme-catalyzed reactions are especially sensitive to small changes in temperature. Because of this, the metabolism of *ectotherms*, organisms whose internal body temperature is determined by their surroundings, are often determined by the surrounding temperature.

PURPOSE

The purpose of this experiment is to determine the effect of temperature changes on the aerobic respiration of yeast.

EQUIPMENT/MATERIALS

LabPro	250-mL beaker
TI Graphing Calculator	two 600-mL beakers (for water baths)
Vernier Dissolved Oxygen Probe	10-mL graduated cylinder
DO electrode filling solution and 1-mL pipet	100-mL
Sodium sulfite calibration solution	warm and cool water
Calibration bottle	wash bottle with distilled water
thermometer	hot plate
2 large test tubes	1% glucose solution
2 small test tubes	yeast solution

SAFETY

- Always wear an apron and goggles in the lab.

PRE-LAB PROCEDURE

Important: Prior to each use, the Dissolved Oxygen Probe must warm up for a period of 10 minutes as described below. If the probe is not warmed up properly, inaccurate readings will result. Perform the following steps to prepare the Dissolved Oxygen Probe.

1. Prepare the Dissolved Oxygen Probe for use (see Figure 1).
 - a. Unscrew the membrane cap from the tip of the probe.
 - b. Using a pipet, fill the membrane cap with 1 mL of DO Electrode Filling Solution.
 - c. Carefully thread the membrane cap back onto the electrode.
 - d. Place the probe into a 250-mL beaker containing distilled water.

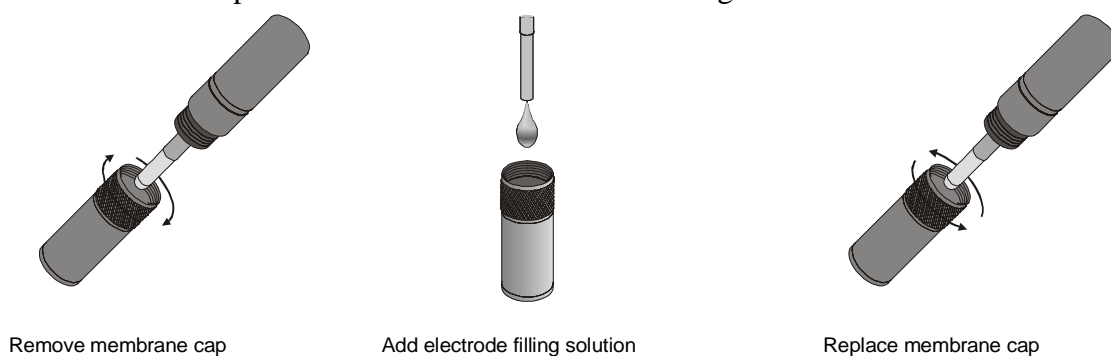


Figure 1

2. Plug the Dissolved Oxygen Probe into Channel 2 of the LabPro. Use the link cable to connect the TI Graphing Calculator to the interface. Firmly press in the cable ends.
3. Turn on the calculator and start the DATAMATE program. Press to reset the program.

4. Set up the calculator and interface for the Dissolved Oxygen Probe.
 - a. If the calculator displays DO (MG/L) in CH 1, proceed to Step 5. If it does not, continue with this step to set up your sensor manually.
 - b. Select SETUP from the main screen.
 - c. Press to select CH1.
 - d. Select D. OXYGEN (MG/L) from the SELECT SENSOR menu.
 - e. Select OK to return to the main screen.
5. It is necessary to warm up the Dissolved Oxygen Probe for 10 minutes before taking readings. With the probe still in the distilled water beaker, wait 10 minutes while the probe warms up. The probe must stay connected at all times to keep it warmed up.
6. Prepare the calculator and interface for calibration.
 - a. Select SETUP from the main screen.
 - b. Press once to select CH2.
 - c. Select CALIBRATE from the setup screen.
7. Calibrate the Dissolved Oxygen Probe.
 - If your instructor directs you to manually enter the calibration values, select MANUAL ENTRY from the CALIBRATION screen. Enter the slope and intercept values, select OK, then proceed to Step 1 of the procedure.
 - If your instructor directs you to perform a new calibration, select CALIBRATE NOW from the CALIBRATION screen and follow this procedure.

Zero-Oxygen Calibration Point

- a. Remove the probe from the water bath and place the tip of the probe into the Sodium Sulfite Calibration Solution (see Figure2).

Important: No air bubbles can be trapped below the tip of the probe or the probe will sense an inaccurate dissolved oxygen level. If the voltage does not rapidly decrease, tap the side of the bottle with the probe to dislodge the bubble. The readings should be in the 0.2- to 0.5-V range.
- b. When the voltage stabilizes (~1 min), press .
- c. Enter "0" as the known value in mg/L.

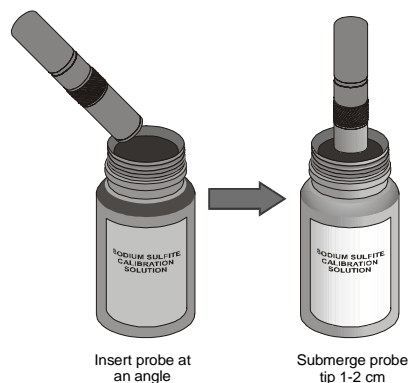
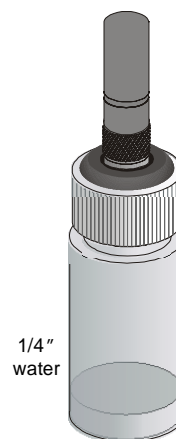


Figure 2

Saturated DO Calibration Point

- a. Rinse the probe with distilled water.
 - b. Unscrew the lid of the calibration bottle provided with the probe. Slide the lid and the grommet about 1/2 inch onto the probe body.
 - c. Add water to the bottle to a depth of about 1/4 inch and screw the bottle into the cap, as shown. **Important:** Do not touch the membrane or get it wet during this step (see Figure 3).
 - d. Keep the probe in this position for about a minute. The readings should be in the 2.0- to 3.3-V range. When the voltage stabilizes, press .
 - e. Enter the correct saturated dissolved-oxygen value (in mg/L) from Table 3 (for example, "8.66") using the current barometric pressure and air temperature values. If you do not have the current air pressure, use Table 4 to estimate the air pressure at your altitude.
 - f. Select OK to return to the setup screen.
 - g. Select OK to return to the main screen.
 - h. Return the Dissolved Oxygen Probe to the distilled water beaker.
8. Set up the data-collection mode.
- a. To select MODE, press (the up arrow key) once and press .
 - b. Select TIME GRAPH from the SELECT MODE menu.
 - c. Select CHANGE TIME SETTINGS from the TIME GRAPH SETTINGS menu.
 - d. Enter "2" as the time between samples in seconds.
 - e. Enter "60" as the number of samples (data will be collected for 2 minutes).
 - f. Select OK to return to the setup screen.
 - g. Select OK to return to the main screen.

**Figure 3****PROCEDURE**

1. Prepare a warm and a cool water bath. A water bath is simply a large beaker of water at a specific temperature. Obtain a 600-mL beaker and place about 200 mL of room temperature water in it. The water temperature should be in the range of 20 – 25°C. Obtain a second 600-mL beaker and place about 150 mL of water in it. Add enough ice to adjust the temperature of the water bath to 10°C.
2. Obtain two clean, small test tubes. Label them "1" and "2". Place 2 mL of the yeast solution into each test tube.

3. Obtain two clean, large test tubes and label them “A”, and “B”. Place 20 mL of the glucose solution into each test tube.
4. Place test tube 1 and test tube A in the warm water bath for 5 minutes. Place test tube 2 and test tube B in the cool water bath for 5 minutes.
5. If the calculator screen has gone blank, press the **ON** key to power up the calculator and select YES from the CONTINUE menu.
6. After test tube 1 and test tube A reach the temperature of the water bath,
 - a. cover the top of the test tube and vigorously shake the contents of test tube A for about 2 minutes to aerate the solution.
 - b. gently mix test tube 1 to suspend the yeast.
 - c. add the yeast solution from test tube 1 to test tube A.
 - d. place test tube A back into the water bath.
 - e. place the thermometer into test tube A and check the temperature. Record the initial temperature in Table 1.
7. Remove the thermometer and place the Dissolved Oxygen Probe into test tube A. Gently and continuously stir the solution with the probe. **Note:** You must stir the probe in the solution for the entire data collection period. The test tube should remain in the water bath during data collection.
8. After stirring the solution for 20 seconds, select START on the calculator to begin data collection. Data collection will end after 2 minutes. **Note:** When data collection has finished, a graph of DO VS. TIME will be displayed.
9. Remove the Dissolved Oxygen Probe from the solution and quickly measure the temperature of the yeast solution with the thermometer. Record the final temperature in Table 1.
10. Rinse the Dissolved Oxygen Probe with water and place it back in the beaker of distilled water.
11. Press **ENTER** on the calculator to return to the main screen.

12. Perform a linear regression to calculate the rate of respiration.
 - a. Select **4:ANALYZE** from the main screen.
 - b. Select **2:CURVE FIT** from the ANALYZE OPTIONS menu.
 - c. Select **1:LINEAR (CH 1 VS TIME)** from the CURVE FIT menu.
 - d. The linear-regression statistics for these two lists are displayed for the equation in the form:

$$Y=a*x+b$$

- e. Enter the slope, a , as the rate of respiration for the temperature tested in Table 1.
 - f. Press to view a graph of the data and the regression line.
 - g. Press to return to the ANALYZE menu.
 - h. Select **1:RETURN TO MAIN SCREEN** from the ANALYZE menu.
13. Repeat Steps 6 – 12, using test tubes “2” and “B”, which are in the cool water bath in place of test tubes “1” and “A”. Be sure the temperatures of the water bath and both solutions have equilibrated. **Note:** One of the team members may want to start Step 14 at this time.
14. Prepare two more water baths, one at 40°C and one at 60°C.
15. Clean out the test tubes and refill with yeast solution and glucose solution as instructed in Steps 2 – 3.
16. Repeat Steps 6 – 12, using test tubes “1”, “A” and the water bath at 60°C.
17. Repeat Steps 6 – 12, using test tubes “2”, “B” and the water bath at 40°C.
18. If time permits,
 - Prepare two more water baths, one at 30°C and one at 50°C.
 - Repeat Steps 6 – 12, using test tubes “1”, “A” and the water bath at 50°C.
 - Repeat Steps 6 – 12, using test tubes “2”, “B” and the water bath at 30°C.
19. Clean all of the glassware and probes. Place the Dissolved Oxygen Probe back in the beaker of distilled water.

DATA SHEET

Name _____

Name _____

Period _____ Class _____

Date _____

AEROBIC RESPIRATION

DATA TABLES

Table 1			
Initial Temperature (°C)	Final Temperature (°C)	Average Temperature (°C)	Rate of Respiration (mg /L/s)

Table 2: Class Data		
Assigned Temperature (°C)	Temperature (°C)	Rate of Respiration (mg /L/s)
10		
20		
30		
40		
50		
60		

5. What will happen to the yeast after the oxygen concentration in the solution drops to zero? Why will they continue to respire sugar? Explain.

EXTENSIONS

1. Design an experiment to measure and compare the effect of other environmental conditions on the respiration rate of yeast.
2. Design an experiment to measure and compare the respiration rate of a different aquatic organism at different temperatures. What changes to this experimental procedure might be necessary?
3. Carry out the experiment you designed in Step 1 or Step 2.
4. Compare the initial concentration of dissolved oxygen you measured at every temperature. What trends do you see? How does this compare to what you expected? Explain.

Table 3: 100% Dissolved Oxygen Capacity (mg/L)												
	770 mm	760 mm	750 mm	740 mm	730 mm	720 mm	710 mm	700 mm	690 mm	680 mm	670 mm	660 mm
0°C	14.76	14.57	14.38	14.19	13.99	13.80	13.61	13.42	13.23	13.04	12.84	12.65
1°C	14.38	14.19	14.00	13.82	13.63	13.44	13.26	13.07	12.88	12.70	12.51	12.32
2°C	14.01	13.82	13.64	13.46	13.28	13.10	12.92	12.73	12.55	12.37	12.19	12.01
3°C	13.65	13.47	13.29	13.12	12.94	12.76	12.59	12.41	12.23	12.05	11.88	11.70
4°C	13.31	13.13	12.96	12.79	12.61	12.44	12.27	12.10	11.92	11.75	11.58	11.40
5°C	12.97	12.81	12.64	12.47	12.30	12.13	11.96	11.80	11.63	11.46	11.29	11.12
6°C	12.66	12.49	12.33	12.16	12.00	11.83	11.67	11.51	11.34	11.18	11.01	10.85
7°C	12.35	12.19	12.03	11.87	11.71	11.55	11.39	11.23	11.07	10.91	10.75	10.59
8°C	12.05	11.90	11.74	11.58	11.43	11.27	11.11	10.96	10.80	10.65	10.49	10.33
9°C	11.77	11.62	11.46	11.31	11.16	11.01	10.85	10.70	10.55	10.39	10.24	10.09
10°C	11.50	11.35	11.20	11.05	10.90	10.75	10.60	10.45	10.30	10.15	10.00	9.86
11°C	11.24	11.09	10.94	10.80	10.65	10.51	10.36	10.21	10.07	9.92	9.78	9.63
12°C	10.98	10.84	10.70	10.56	10.41	10.27	10.13	9.99	9.84	9.70	9.56	9.41
13°C	10.74	10.60	10.46	10.32	10.18	10.04	9.90	9.77	9.63	9.49	9.35	9.21
14°C	10.51	10.37	10.24	10.10	9.96	9.83	9.69	9.55	9.42	9.28	9.14	9.01
15°C	10.29	10.15	10.02	9.88	9.75	9.62	9.48	9.35	9.22	9.08	8.95	8.82
16°C	10.07	9.94	9.81	9.68	9.55	9.42	9.29	9.15	9.02	8.89	8.76	8.63
17°C	9.86	9.74	9.61	9.48	9.35	9.22	9.10	8.97	8.84	8.71	8.58	8.45
18°C	9.67	9.54	9.41	9.29	9.16	9.04	8.91	8.79	8.66	8.54	8.41	8.28
19°C	9.47	9.35	9.23	9.11	8.98	8.86	8.74	8.61	8.49	8.37	8.24	8.12
20°C	9.29	9.17	9.05	8.93	8.81	8.69	8.57	8.45	8.33	8.20	8.08	7.96
21°C	9.11	9.00	8.88	8.76	8.64	8.52	8.40	8.28	8.17	8.05	7.93	7.81
22°C	8.94	8.83	8.71	8.59	8.48	8.36	8.25	8.13	8.01	7.90	7.78	7.67
23°C	8.78	8.66	8.55	8.44	8.32	8.21	8.09	7.98	7.87	7.75	7.64	7.52
24°C	8.62	8.51	8.40	8.28	8.17	8.06	7.95	7.84	7.72	7.61	7.50	7.39
25°C	8.47	8.36	8.25	8.14	8.03	7.92	7.81	7.70	7.59	7.48	7.37	7.26
26°C	8.32	8.21	8.10	7.99	7.89	7.78	7.67	7.56	7.45	7.35	7.24	7.13
27°C	8.17	8.07	7.96	7.86	7.75	7.64	7.54	7.43	7.33	7.22	7.11	7.01
28°C	8.04	7.93	7.83	7.72	7.62	7.51	7.41	7.30	7.20	7.10	6.99	6.89
29°C	7.90	7.80	7.69	7.59	7.49	7.39	7.28	7.18	7.08	6.98	6.87	6.77
30°C	7.77	7.67	7.57	7.47	7.36	7.26	7.16	7.06	6.96	6.86	6.76	6.66

Table 4: Approximate Barometric Pressure at Different Elevations					
Elevation (feet)	Pressure (mm Hg)	Elevation (feet)	Pressure (mm Hg)	Elevation (feet)	Pressure (mm Hg)
0	760	2000	708	4000	659
250	753	2250	702	4250	653
500	746	2500	695	4500	647
750	739	2750	689	4750	641
1000	733	3000	683	5000	635
1250	727	3250	677	5250	629
1500	720	3500	671	5500	624
1750	714	3750	665	5750	618

