Metabolism and Oxygen Consumption in Aquatic Organisms

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Background Information

I use this lab in a low enrollment junior/senior level animal physiology course. We also examine the terrestrial metabolism/oxygen consumption of lizards and mice using small animal chambers. The students are encouraged to work in groups and develop their own hypotheses. I have also used this technique in student research projects. Publications from student research projects are included in the References. Please contact me if you would like more information.

I have used aquatic, adult red-spotted newts, tadpoles, and goldfish with my classes. You need to adjust the volume of the jars and determine if there will be enough oxygen consumption during the lab period. You can always increase the temperature of the water to increase the oxygen consumption rate. At least a 5-10% difference in the oxygen concentrations between the control and experimental jars is desired so the data will be reliable. Students can become very proficient with this technique. Our data usually compares well with published data.

Because the amount of activity can vary with different organisms, one of the participants in the workshop suggested that you could add a slowly turning stir bar to the closed jar so that the fish will orient to this current. You could also increase the rate of stirring to investigate effects of increased exercise. I have used a very slow shaking water-bath with adult, aquatic red-spotted newts to provide a gentle current so an oxygen deficient water layer did not develop next to the skin of these animals that rely on cutaneus respiration when submerged.

Check with your school about using vertebrates. The animals are not harmed unless left too long in the closed jars. If the animals are in the same water too long, they will run out of oxygen and you will not have an accurate relationship between oxygen consumption and metabolism. Sarah Deel from Carlton College shared that there is an oxygen consumption lab for a sea anemone available through Research Link 2000. Go to www.ResearchLink.ferris.edu; choose Sea Anemone; and then go to Instructional Lab Exercise: Energy Utilization.

Student Handout

Objectives:

1. To learn the Micro-Winkler determination of dissolved oxygen in water
2. To investigate metabolic rates of aquatic organisms
3. To compare data with published values.

Introduction

Metabolism can be defined as all of the chemical changes in an organism. This lab is concerned with only energy metabolism, i.e., reactions involved in the production and utilization of energy. We will measure oxygen consumption to estimate energy metabolism because the rate of oxygen consumption and energy utilization are directly related under most circumstances.

In this exercise you will determine the oxygen consumption of aquatic organisms. This lab describes the technique of determining the oxygen in small volumes of water. The Winkler determination of dissolved oxygen is a titration based on the oxidizing properties of dissolved oxygen (DO). A divalent manganese solution is added to the sample followed by a strong alkali. The DO rapidly oxidizes an equivalent amount of the divalent manganous hydroxide precipitate to hydroxides of a higher valency state. When you add an acidic solution in presence of iodide ions, the oxidized manganese reverts to the divalent state, with the release of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate. With a starch indicator, the end point is a clear solution. You can then calculate the amount of DO in the original sample.

$$MnSO_4 + 2\ KOH = Mn(OH)_2 + K_2SO_4$$
$$4\ Mn(OH)_2 + 2H_2O + O_2 = 4\ Mn(OH)_3$$
$$2\ Mn(OH)_3 + 2\ K^+ + 2\ I^- + 2\ H_3PO_4 = 2\ Mn^{++} + 2\ K^+ + 2\ PO_4^{-3} + I_2 + 6\ H_2O$$
$$I_2 + 4\ Na^+ + 2\ S_2O_3^{-2} = 4\ Na^+ + 2\ I^- + S_4O_6^{-2}$$

Winkler determinations are done routinely in testing water samples. We are using a modification called the Micro-Winkler that requires only 10 mL of sample (Burke, 1962). Take at least two samples every time you determine the DO.

What are some of the hypotheses that you might investigate with this technique? What variables do you need to control if you can? Where will you find comparable published data? I suggest that you prepare a lab plan with data tables before you begin this lab to help you make effective use of your time.

Preparation of closed respirometers

1. Weigh the dry jar and lid. Record the weight. Fill a jar with dechlorinated water. Weigh the organism, and put it in water. Seal the jar so there are **no bubbles** and weigh the filled jar with an organism. If you fill the jar under water, it is easy to eliminate bubbles.
2. There must be at least two empty jars for every 4 animals. These control jars should be treated in the same way so you can compare amounts of oxygen dissolved in water with and without an animal.
3. Put the jar with an organism into a constant temperature environment. Record the time to start.
4. Determine the temperature of the water.
5. Observe the behavior of the organisms periodically. Can you observe any ventilatory movements? Can you calculate the ventilatory rate?

While you are waiting for the organism to use up oxygen in the jar, read the directions for Micro-Winkler test and practice the test with the water that you used to fill the jars. Ask your instructor for the amount of oxygen in water at the temperature of your water.

Directions for Determining the Oxygen Consumption of Aquatic Organisms using the MicroWinkler Method

1. Put about 1 mL of manganous sulfate and 1 mL alkaline iodide solutions in separate wells of a glazed porcelain depression plate.
2. Draw up 10 mL of sample into a 10 mL syringe (no needle). Invert the syringe and expel water to the 9.4 mL mark taking care that there are no bubbles. If you have a bubble, try tapping the syringe to remove the bubble.
3. Draw up 0.2 mL of manganous sulfate into the syringe. Wipe off and seal the end of the syringe with Parafilm. Mix by inverting the syringe 10 times while holding Parafilm on the syringe tip.
4. Draw up 0.2 mL of alkaline iodide. Wipe off and seal the end of the syringe with Parafilm. Mix by inverting the syringe 10 times while holding Parafilm on the tip.

BE VERY CAREFUL OF CONCENTRATED SULFURIC ACID. KEEP THE ACID BOTTLE IN FINGER BOWL TO CATCH DRIPS. USE THE AUTOMATIC PIPETTOR.

5. Add 0.2 mL of sulfuric acid to small (25 - 50 mL) Erlenmeyer flasks that have stir bars in the bottom. Use the Selectapette Pipetter System or other pipettor to do this so that spilling is minimized.

6. Gently expel the contents of the syringe into the Erlenmeyer flask. Place the flask on a magnetic stirrer or manually swirl the contents of the flask.
7. Titrate to a straw color with 0.0109 N sodium thiosulfate using a 1 mL tuberculin syringe graduated in 0.01 mL. The needle should be on the syringe. When the solution is straw colored (pale yellow) then add 2 drops of starch. Continue to titrate until the solution is clear. Record the volume of sodium thiosulfate that you used.

Calculations (Read before you begin to work.)

We are going to express the oxygen consumption as mL of oxygen consumed per gram of organism per hour so that we can compare our data with published values.

Determine the amount of oxygen in water

Explanation of the Sodium Thiosulfate Correction Factor

0.0109 m.eq. sodium thiosulfate/mL X 8mg O_2/m.eq. sodium thiosulfate = 0.0872 mg O_2/L (ppm) of sodium thiosulfate

[(0.0872 mg O_2/mL sodium thiosulfate)/9.4 mL] X $\frac{1000 mL}{L}$) = 9.276 mg/L (ppm) O_2 per mL of sodium thiosulfate

If you multiply the mL of sodium thiosulfate used in the titration by 9.28 you will get the mg/L O_2. Take the mean of the mL of sodium thiosulfate used to titrate each sample then multiply by 9.28 to get the mg/L or mg of oxygen per liter of solution.

Multiplying by 0.7 converts mg/L to mL/L or μL/mL (1 L of O_2 = 1429 mg O_2, Prosser, 1973, p.166)

Oxygen Consumption Calculations

Calculation of Volume of water in flask

[Weight of full jar with an organism] - [Weight of empty jar and its cover] - [weight of the organism] = weight of water. Assume 1 gram of water = 1 mL of water.

Calculation of O_2 consumed

Net mg/L used in jar (Mean mg/L of blanks - mg/L of experimental) X 0.7 X mL volume in jar = mL/L of O_2 consumed.

O_2 consumed /unit time X mass or mL O_2/gram hour

Record the time you begin and end your experiment. The end is just before you sample for Micro-Winkler. Express time in **decimals**; i.e. 1 hour 15 minutes = 1.25 hour

The weight of the organism can be determined before or after the procedure depending on your experimental design.

Divide the mL of oxygen consumed by the time and weight of the organism = mL O_2/gh

How does your data compare with the literature values?

Sample calculation:

Filled water and fish	164.20 g	Start Time	2:20 p.m.
Minus Initial wt jar and lid	127.8 g	End time	3.33 p.m.
Minus wt fish	2.07 g	Total time	1.22 hour
Wt (mL) of water	34.27 mL		

Jar 1 with fish

1.13 mL sodium thiosulfate
X 9.28 = 10.48 mg/L oxygen

Jar 2 no fish

1.00 mL sodium thiosulfate
X 9.28 = 9.28 mg/L oxygen

[10.48 mg/L – 9.28 mg/L] X 0.7 X 34.27 mL = 28.8 µL oxygen consumed

28.8 µL oxygen /2.07 g of fish/1.22 hours = 11.4 µL/g·hour or 0.011 mL/g·hour

Table 1. Examples of class data and literature values (* from Prosser, 1973).

	Mass (g)	Temp (C^o)	mL O_2/g·hour
Goldfish	0.5	22.2	0.281
Goldfish	2	21.7	0.184
Goldfish	1.35	22.2	0.414
Goldfish	?	25	0.189
Bullfrog tad	?	21	0.618
Bullfrog tad	?	30	0.035
*Rana tadpole	213	15	0.007
*Rana tadpole	213	20	0.065
*Carassius	33	15	0.077

References

Burke, J. D. 1962. Determination of oxygen in water using a 10-mL syringe. Journal of the Elisha Mitchell. Science Society. 78: 145-147.

Pitkin, R. B. 1987. Anemia and cutaneous gas exchange in adult aquatic red-spotted newts. *Notophthalmus viridescens viridescens*. Journal of Herpetology. 21(1):1-5.

Pitkin, R. B. and C. L. Snyder. 1990. Effects of anemia, temperature, and activity on the oxygen consumption of *Rana catesbeiana* tadpoles. Journal of the Pennsylvania Academy of Science. 64(3): 111-115.

Prosser, C. L. 1973. Comparative Animal Physiology. Third Edition. W. B. Saunders, Philadelphia.

Materials

Solutions needed:

- Manganous sulfate (Hach Cat. #275)
- Alkaline-iodide-azide reagent (Hach Cat. #277)
- Sulfuric Acid Concentrated; place bottle in fingerbowl
- Sodium thiosulfate standard solution, stabilized, 0.0109N (Hach Cat. #24089-11)
- Starch Indicator Solution (Hach Cat. #349-37)

For a lab with 5 groups:

- About 20 jars with tightly sealing lids or rubber stoppers
- Balances
- 5 Porcelain spot plates
- Syringes 10-mL B-D (Ward's 14 W1618) and 1-mL B-D Tuberculin (with needles 26g 3/8 inch)
- Automatic pipettors (100 -1000 uL) with blue pipette tips or plastic transfer pipettes
- Kim wipes
- Parafilm
- Paper towels
- Selectapette Pipetter System (Fisher Cat. No. 21-260)
- Timers
- Aquarium with aged tap water and aerator
- 5 magnetic stirrers and 10 small stir bars
- 5 Scissors/Parafilm
- Water bath to increase temperature
- Fish, salamanders, larval salamanders, tadpoles

Hach Company
P.O. Box 389
Loveland, CO 80539-0389
800 227-4224

Handling and Disposal notes:

The Material Data Safety Sheets say to avoid contact with skin, eyes and clothing for Alkaline-Iodide-Azide which is the most toxic of the chemicals in this lab. Students should wear gloves and goggles. Sodium thiosulfate and manganous sulfate can be poured down the drain with excess water. Alkaline-Iodide-Azide Reagent should be burned in a chemical incinerator equipped with an after burner and scrubber. We have a hazardous waste disposal company pick this up.