Core practical 9: Investigate factors affecting the rate of aerobic respiration using a respirometer

Objectives

- To understand how to use a respirometer
- To be able to interpret and evaluate respirometer data

Safety

- Soda lime is corrosive. Wear eye protection. Do not touch the soda lime; use a spatula to position in the respirometer. Do not inhale any dust.
- Wash your hands after handling organisms.

Specification links

- Practical techniques 1, 2, 3, 8, 12
- CPAC 1a, 2a, 2b, 4a, 4b

Procedure

1. Assemble the respirometer (see fig A). Check that you know how to use it, especially the operation of the three-way tap. Clamp the syringe and respirometer in position when in use.
2. Place a known mass of one type of organism into the boiling tube, and replace the bung. Record the mass. Handle live animals with care to avoid harming them.
3. Place a drop of coloured fluid at the open end of the glass tube using a dropping pipette. Open the connection between the syringe and the respirometer. Use the syringe to draw the fluid onto the scale at the end furthest from the respirometer.
4. Mark the starting position of the fluid on the glass tube or note the reading.
5. Close the tap to isolate the respirometer from the atmosphere and the syringe and start the stop clock immediately.
6. Note the position of the fluid at 1 minute intervals for 5 minutes.
7. Work out the distance travelled by the liquid during each minute. Record your results in a suitable table. Include columns for the mean rate of oxygen uptake during the 5 minutes and the mean rate per gram of organism.
8. At the end of 5 minutes, open the connection to the outside air. If time is available, repeat the process for a different organism.

Notes on procedure

- If three-way taps are used, it will be necessary to demonstrate their use. It would be of benefit to demonstrate other respirometers including U-tube (Dixon–Barcroft) types.
- This investigation has been organised to minimise the number of respirometers that are required. If respirometers are not in short supply, students could set up and run a suitable control respirometer alongside the test and use the results to control for any changes in air temperature or pressure.
- Using the same mass of different organisms may be not be possible because of the very different relative masses of, for example, woodlice and peas. Students should realise that as long as the mass is recorded, comparisons per gram can be made.
- The time (5 minutes) for the experiment may need to be adjusted if respiration rates are very fast or very slow. If the fluid does not move, check that all seals are airtight; a little petroleum jelly may help to seal them. Equipment should be sealed firmly but over-tightening can cause breakages and injury.
- Point out to students that NaOH or KOH solution could be used instead of the soda lime. While these are more reliable in terms of CO_2 absorption, they are also more difficult and less safe to work with.

Answers to questions

1. Both plants and animals respire to produce ATP for cellular processes but animals also move around and require additional ATP for muscle contraction. Respiration rates are therefore usually higher in animals.
2. Some variables may not have been effectively controlled, such as temperature, amount of movement, age or stage of development of organisms. The low resolution of the manometer scale will also cause some uncertainty in readings.
3. Temperature could be controlled using a water bath. Organisms could be matched between groups for size or stage of development.
4.
   (a) A decrease in temperature or an increase in atmospheric pressure would cause movement towards the respirometer. An increase in temperature or a decrease in atmospheric pressure would cause movement in the opposite direction.
   (b) Movement towards the control respirometer would be subtracted from results; movement away from the control respirometer would be added to results.
5. Soda lime is used to absorb any carbon dioxide produced by the respiring organisms. The gas volume will reduce as oxygen is removed for respiration.
6. A reduction in gas volume will reduce the pressure inside the tube. As it becomes lower than atmospheric pressure, the fluid bubble will move towards the respirometer chamber.

Sample data

Replicate	Distance moved by manometer fluid in 1 minute/mm			
	Woodlice (1 g)	Woodlice control	Peas (5 g)	Peas control
1	5	−1	7	0
2	4	0	5	0
3	6	1	9	1
4	4	0	7	0
5	5	0	7	0

table A Results from a respirometer with capillary tubing of 1 mm internal diameter. Movements in the direction expected from oxygen uptake are positive, while movement in the opposite direction (away from the respirometer) is shown as negative figures.

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All the maths you need

- Recognise and make use of appropriate units in calculations.
- Use an appropriate number of significant figures.
- Find arithmetic means.
- Plot two variables from experimental or other data.
- Calculate the circumferences, surface areas and volumes of regular shapes.

Equipment

- respirometer
- live animals such as woodlice or maggots
- actively respiring germinating seeds such as mung beans, peas or other seeds
- soda lime in muslin
- coloured manometer fluid
- spatula
- stop clock
- clamp and stand
- dropping pipette
- mass balance
- eye protection
- fine marker pen or chinagraph pencil

Diagram

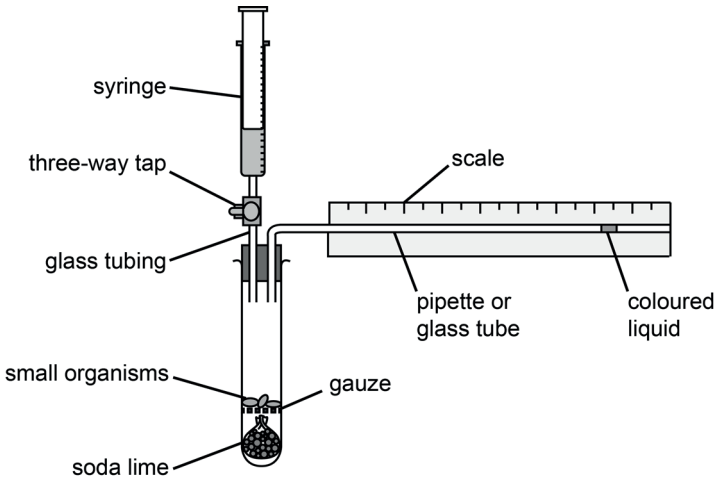

fig A Simple respirometer.

Procedure

1. Assemble the respirometer (see fig A). Check that you know how to use it, especially the operation of the three-way tap. Clamp the syringe and respirometer in position when in use.
2. Place a known mass of one type of organism into the boiling tube, and replace the bung. Record the mass. Handle live animals with care to avoid harming them.
3. Place a drop of coloured fluid at the open end of the glass tube using a dropping pipette. Open the connection between the syringe and the respirometer. Use the syringe to draw the fluid onto the scale at the end furthest from the respirometer.
4. Mark the starting position of the fluid on the glass tube or note the reading.
5. Close the tap to isolate the respirometer from the atmosphere and the syringe and start the stop clock immediately.
6. Note the position of the fluid at 1 minute intervals for 5 minutes.
7. Work out the distance travelled by the liquid during each minute. Record your results in a suitable table. Include columns for the mean rate of oxygen uptake during the 5 minutes and the mean rate per gram of organism.
8. At the end of 5 minutes, open the connection to the outside air. If time is available, repeat the process for a different organism.

Analysis of results

1. If your respirometer does not have volumes marked onto it, convert the distance moved by the liquid into the volume of oxygen used. Use the formula for the volume of a cylinder: volume $= \pi r^2 h$ where r is the radius of the hole in the glass tube and h is the distance moved.
2. Calculate the mean rate of oxygen uptake during the 5 minutes, then divide by the mass of organisms used to find the mean rate per gram of organism ($mm^3\ min^{-1}\ g^{-1}$).
3. Collect mean results for the rate of oxygen uptake per gram from other groups in the class. You could collect the results in a spreadsheet. Calculate the overall mean for each organism. If there are sufficient data, calculate the standard deviation.
4. Plot a suitable graph of the class results. Use the range or standard deviation to indicate the precision of the data.
5. Comment on your results and the variability of the data.

Learning tip

- A respirometer measures oxygen uptake by respiring organisms. Any CO_2 produced is absorbed in the apparatus, so any change in gas volume is due to removal of oxygen by the organisms through aerobic respiration. Hence oxygen uptake is used as an indication of respiration rate.

Questions

1. Consider your results and any differences in rates of oxygen uptake between different types of organism. Animals usually have a higher respiration rate per gram than plants. Explain this difference.
2. Suggest what factors may have caused any variability seen in class results.
3. How could this variability be reduced and the precision of the results improved?
4. It would have been better to have used a control respirometer alongside the experimental set-up. In the control the equipment is the same but the organisms are replaced by non-living material such as glass beads.
   (a) Explain what may cause the liquid in the control tube to move towards and away from the respirometer.
   (b) Explain how you would use the control results to correct your experimental data.
5. What is the importance of using soda lime in the respirometer? How does this affect the volume of gas in the apparatus?
6. How does this influence the movement of the liquid in the capillary tube?

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- Soda lime is corrosive. Wear eye protection. Avoid skin contact; wear protective gloves. Avoid inhaling dust. Make up the muslin bags in a fume cupboard or a well-ventilated area.
- Wash your hands after handling organisms.

Equipment per student/group	Notes on equipment
respirometer	There are many designs of respirometer. Fig A on the Student sheet shows a relatively simple one. A three-way tap allows easy positioning of manometer fluid but can be replaced with a clip and rubber tube if not available. A U-tube (Dixon–Barcroft) respirometer would be useful, even if only used for demonstration. The metal gauze should sit snugly in the tube but should not fit too tightly. The bore of the glass tubing should be known; 1 mm tubing works well.
live animals such as woodlice or maggots	Approximately 5 g per group. Maggots can be sourced from a fishing shop or bought online. They can be kept in the fridge for a couple of weeks. Weighing the animals out into tubes in advance can help prevent escapes in the classroom but students should be able to record the mass of organism in each tube.
actively respiring germinating seeds such as mung beans, peas or other seeds	5 g per group. These must be soaked for 12–24 hours before the investigation to allow germination to begin. The mass of organisms may need to be adjusted depending on respirometer size and the density of the organism.
soda lime in muslin	Approximately 20 g per group. Place the soda lime onto the centre of a piece of muslin and tie with cotton or a small cable tie. Soda lime can be placed directly into the bottom of the respirometer, but wrapping it in muslin reduces the likelihood of organisms coming into contact with the dust and reduces the risk to students when handling it.
coloured manometer fluid	Provide about 2 cm^3 per group. Use food dye in water. Add a drop of washing up liquid to reduce adherence to the glass tubing.
spatula	One per group
stop clock	One per group
clamp and stand	One per group
dropping pipette	One per group

mass balance	These are needed if students are to weigh their own organisms.
eye protection	One per group
fine marker pen or chinagraph pencil	One per group

Notes