

Volumetric measurement of breathing in small animals (Item No.: P4090100)

Curricular Relevance



Keywords:

Oxygen consumption, Volumetric measurement, Respirometer, Carbon dioxide consumption, Compensation vessel, Q10 value

Overview

Principle

Measure the oxygen consumption of insects in relation to ambient temperature and body weight. Volumetric measurement of the oxygen consumption of an animal enclosed in a temperature-controlled spirometer. Absorption of the exhaled carbon dioxide using concentrated potassium hydroxide solution.



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Student's Sheet

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Equipment

Position No.	Material	Order No.	Quantity
1	Respirometer, complete assembly	65998-00	1
2	Immersion thermostat Alpha A, 230 V	08493-93	1
3	Bath for thermostat, makrolon	08487-02	1
4	Support base, variable	02001-00	1
5	Support rod, stainless steel, I = 250 mm, d = 10 mm	02031-00	2
6	Boss head	02043-00	1
7	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
8	Volumetric pipette, 10 ml	36578-00	1
9	Pipettor	36592-00	1
10	Tweezers,straight,pointed,120mm	64607-00	1
11	Glass beads, d 6 mm, 850 pcs.	36756-25	1
12	Silicon grease Molykote, 50 g	31863-05	1
13	Potass.hydroxide,30% sol., 500 ml	48209-50	1
14	Tubing connector, ID 6-10mm	47516-01	2

Tasks

Measure the oxygen consumption of insects in relation to ambient temperature and body weight. Volumetric measurement of the oxygen consumption of an animal enclosed in a temperature-controlled spirometer. Absorption of the exhaled carbon dioxide using concentrated potassium hydroxide solution.



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Set-up and procedure

- The U-shaped manometer of the respirometer (Fig. 2) is filled to a height of approximately 3 cm with manometer fluid (diluted ink solution) using a hypodermic syringe. The needle should be introduced into the U-tube to below the level of the connection hole to the chamber, so that no manometer fluid can enter the chamber during filling (the silicon tubing may be removed from the connector beforehand). If air bubbles are trapped, these must be removed by tapping the respirometer carefully.
- The two troughs are removed from their containers and each filled with 10 ml of 50% potassium hydroxide solution. Caution: potassium hydroxide is highly corrosive – use a pipette bulb. To improve carbon dioxide absorption, strips of filter paper are placed in the troughs with the potassium hydroxide solution.
- When the troughs are replaced in the containers and on subsequent use of the respirometer it must be ensured that the perforated cover plates are not splashed with potassium hydroxide solution: otherwise the experimental animals will be burned.
- A strong hypodermic needle, to which a graduated 1 ml plastic syringe is connected, is passed through one of the two rubber stoppers. The syringe is drawn up to the 1 ml mark. The sides of the two rubber stoppers are slightly greased with stopcock grease. The two pieces of silicon tubing positioned on the tube connectors are fitted with a large pinchcock (Fig. 2), but are not yet clamped off.



- 5 mealworms are weighed and placed in one of the two containers -the measuring chamber -and approximately the same volume of glass beads is placed in the other -the compensation vessel. The measuring chamber is made airtight using the rubber stopper with the hypodermic syringe inserted, and the compensation chamber sealed with the other rubber stopper. The respirometer is then placed carefully in the water bath and secured against accidental movements with clamps and stands (Fig. 1) . The water should be at room temperature (value to be recorded). After an acclimatisation period of 10 minutes the two silicon tubes are clamped off simultaneously using the pinchcock.
- The respirometer is now ready for measurements to be taken. In the measuring chamber, consumption of oxygen combined with the absorption of carbon dioxide leads to a continuous decrease in pressure. The air in the compensating chamber is able to expand and forces the manometer column gradually towards the measuring chamber.
- Every five minutes the plunger of the syringe is depressed until the manometer menisci are again at the same level. The volume of oxygen used in each 5-minute period is read off in ml from the syringe scale and recorded. After one hour the measurements are interrupted and the containers ventilated by careful opening of the pinchcock. Only then can the rubber stoppers be removed and the animals taken out of the measuring chamber.
- After a break of at least one hour the experiment is repeated with a 10 °C increase in bath temperature, using the same 5 mealworms. The experiment may also be repeated with other small animals (e.g. meal beetles).

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Result and evaluation

Results and evaluation

- The measurement of breathing processes, i.e. determination of the consumption of oxygen or release of carbon dioxide, can be carried out manometrically as well as volumetrically. In the manometric method pressure changes are measured at constant volumes, e.g. by the Warburg technique. As far as the volumetric method is concerned, however, changes in volume are measured at constant pressure. In the respirometer the breathing processes are measured volumetrically. Fluctuations in air pressure are equalized by the compensation chamber, and differences in volume avoided by using containers of the same volume with similar contents (animals, glass beads, potassium hydroxide solution).
- To determine oxygen consumption the mean value of the 12 volume measurements is taken for each temperature and each species. The mean values are converted to ml oxygen per hour and g body weight. The oxygen consumption per g body weight is always higher for small animals than for large ones. An animal's oxygen consumption, however, also depends on its state of motion, nutritional status, sex, age, hormonal influences, light and particularly on temperature. In the physiological temperature range the oxygen consumption of poikilothermic organisms normally increases, for a temperature increase of 10 °C, by a factor of two or three (Q₁₀ = 2 to 3).

