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Procedure For Operation of the Gilson Respirometer

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Principle of Measurements

The Gilson respirometer measures gas exchange of materials in a closed system, at a constant temperature. Glass flasks or reaction vessels containing the material under study are connected by a manometer plus micrometer with digital readout.

As the gas volume in the system changes the fluid level in the manometer changes. An increase in gas pressure in the system caused the manometer fluid to rise whereas a decrease in the gas pressure in the system caused the manometer fluid to drop. Adjusting the balancing knob on top of the digital readout results in a variation, increasing or decreasing, in the volume of the manometer. This allows adjustment of the level of the manometer fluid, back to an original reference point. At the same time the digital readout registers the volume changes as microliters of gas increase or decrease. Carbon dioxide is absorbed by addition of a base in the centerwells of certain flasks, therefore only oxygen exchange is measured. In certain other flasks both CO_2 and O_2 exchange are measured. CO_2 evolution is then calculated rather than measured directly.

Procedures for Operation:

Materials for study are placed in the clean, dry flask. Approximately the same amount of materials should be placed in each flask, i.e. grams of material, number of seeds, etc.

An equal amount of distilled water is added to each flask. Enough water should be added to supply the necessary moisture for measurement of desired reactions.

Too much water may retard the reactions being investigated.

Laboratory Exercise

Respiration

Introduction:

Respiration of seeds, small plants or plant parts may be measured and is a useful tool in many areas of physiological research.

Various methods may be used to measure respiration. In this exercise we will use one of the newer, more sophisticated instruments, the Gilson Differential Respirometer.

Respiration of seeds may be measured at different intervals and may be expressed as respiration at specific intervals after the beginning of imbibition. Respiration may be expressed in micro liters of gas exchange per seed per hour or per unit weight of seed. The respiration quotient RQ is the ratio of CO_2 evolved to O_2 consumed or $\frac{CO_2}{O_2} = RQ$.

Procedure:

You will use corn kernels that have been presoaked for 4 to 5 hours so that imbibition is sufficient for respiration to have progressed to the extent that gas exchange is enough for significant measurements. The first 2 to 3 hours after imbibition begins the rate of respiration is low and gas exchange is minimum.

Place 5 corn kernels of lot A in each of 6 flasks. Repeat for Lot B.

In each of 3 flasks within each seed lot add 15% KOH solution and filter paper strips.

Add 1 ml. of distilled water to each flask.

Set up respirometer and allow 30 minutes for equilibration.

Measure respiration for a 30 minute period.

Allow 2 hours for continued respiration. Measure respiration for another 30 minute period.

After each measuring period record readings, calculate O_2 consumption, CO_2 evolution and RQ for each lot of seed on a per seed per hour basis.

A base solution is added to the centerwells of the necessary number of flasks. (In seed respiration studies we use 15% KOH). Centerwells are filled approximately 1/2 full. A strip of filter paper is placed in each well containing base solution to increase the surface area for absorbing CO_2.

Only water is placed in two flasks. These are attached at the first and last positions and used as thermal barometers, and indicate pressure changes in the system other than gas exchange. A very thin film of sealer is applied around the neck of each flask and around the glass connectors on the respirometer. Also the venting plug in the side arms must be sealed.

Flasks are slipped onto the connectors and rotated from side to side several times to complete the seal.

Four springs are needed for each flask. Two hold the flask on the connector and 2 hold the venting plug in the side arm.

Attached flasks are then lowered into the water bath. Adjust index line to the bottom of the manometer fluid meniscus. (Water level in the tank should be about 1 to 1 1/2 inches from the top when flasks are submerged.)

Throw switches for the main heater, stirring motor and shaking motor. After allowing the water bath to reach the desired temperature, turn on the refrigeration motor .. Operate with both heater and refrigeration unit on. Be sure all digital readouts are on the same number.

Measuring Gas Exchange:

Approximately 30 minutes should be allowed for the system to equilibrate. At any intervals after equilibration measurements may be made.

To close the system:

1. Close the gassing manifold valve.

2. Push down on master lever B to close all operating valves.

These 2 steps must be performed in this order only otherwise manometer fluid will be forced out through the gassing manifold. In a short period of time manometer fluid levels will begin to change. Adjust manometer fluid level back to the index line by turning the balancing knob. Adjustments must be made throughout the measuring period. Final adjustments should be made just prior to opening the system.

To open the system:

1. Operating valves are opened first by pushing up on master lever A.

2. Open the gassing manifold valve. These two steps must be performed in this order only otherwise the manometer fluid will be forced out through the gassing manifold.

When the measuring period is completed record reading. Return digital readouts to reference numbers.

Calculations

The 2 thermal barometer readings are averaged. Determine the difference in the average thermal barometer and each digital readout, record with sign + or -, larger or smaller, than the thermal barometer reading.

This difference is then divided by 1/2 the number of seed or 1/2 the weight of the material being measured, etc. This gives a per unit per correction.

Average the 3 oxygen readings (from flask containing base). The sign + or − must be retained with this average oxygen reading.

The reading from CO_2 is then adjusted using the average O_2 reading with each CO_2 reading as follows:

$+O_2$ add	$+O_2$ subtract	$-O_2$ add	$-O_2$ subtract
$-CO_2$	$+CO_2$	$+CO_2$	$-CO_2$

After adjusting each CO_2 reading, average the three adjusted readings. The readings have now been corrected and adjusted to a per unit per hour basis.