10. Respiration Rate of Germinating Seeds

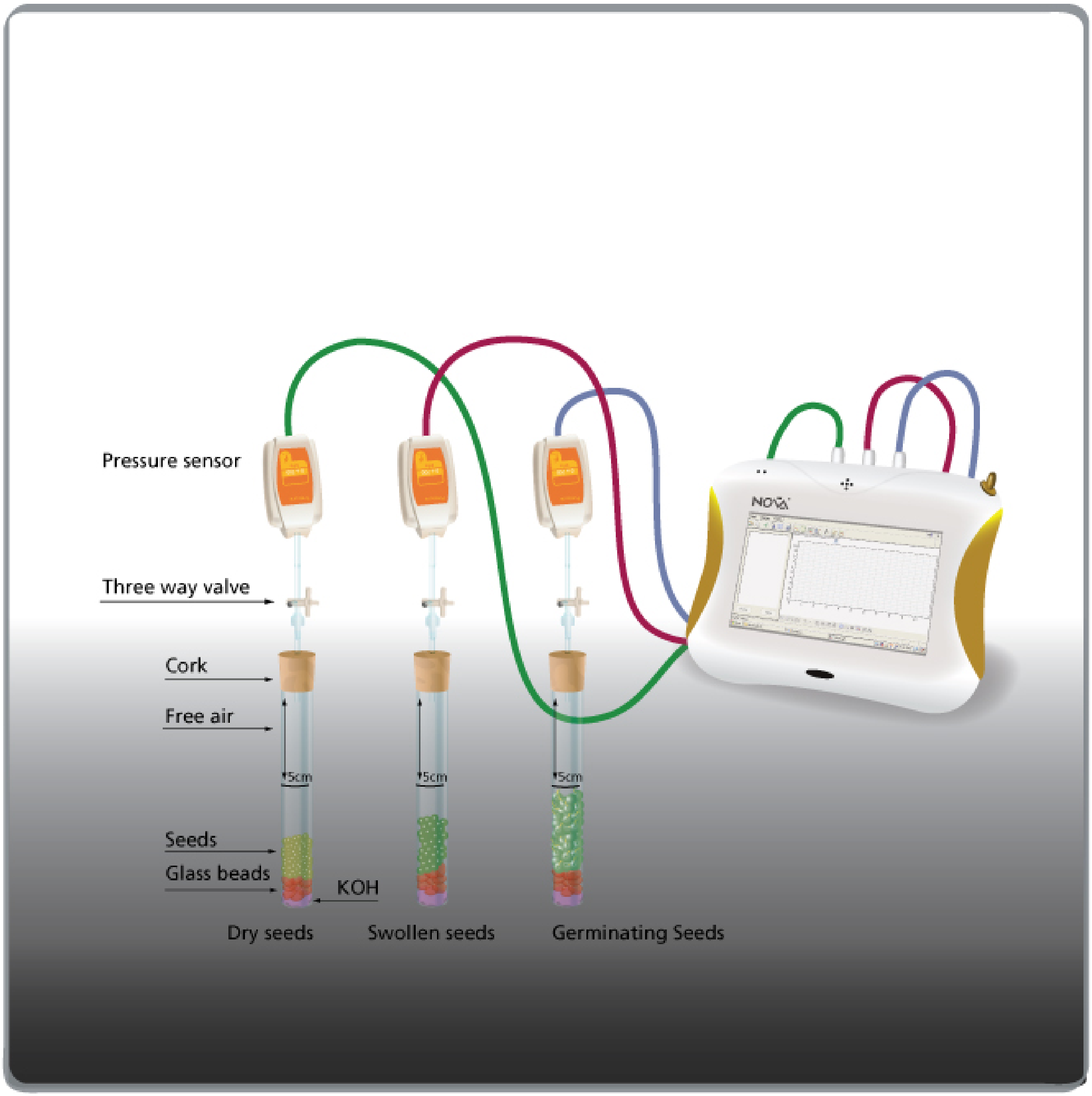


Figure 1

The germination process requires a large amount of energy. Decomposition of storage materials (carbohydrates, lipids and other organic molecules) stored in the seed in the process of cellular respiration, supplies the necessary energy. During respiration, oxygen is consumed while CO_2 is released.

Dry seeds respire at a very low rate. Addition of water to dry seeds first release gases retained in the seeds, a physical process completely unrelated to respiration. But as water content in the seeds increases, the respiration rate is hugely accelerated.

Following consumption of oxygen throughout germination, several stages can be observed: first, the seeds swell as water penetrates into them. At this stage, oxygen consumption increases at a very fast rate.

When the seeds are swollen, roots and shoots start to develop. At this stage the rate of oxygen consumption stabilizes. It increases again as the young sprout continues to grow and the roots and shoots elongate.

Finally, the sprout starts to develop leaves. At this stage most of its storage materials are exhausted and the rate of oxygen consumption decreases.

The rate of the germination process and the rate of respiration depend on abiotic factors including temperature, levels of oxygen and CO_2, exposure to light.

In this experiment the rate of oxygen consumption of germinating seeds will be compared with that of swollen seeds and of dry seeds using Pressure sensors.

KOH is used to remove CO_2 released in the respiration process. CO_2 is heavier than air. It precipitates to the bottom of the test tube and reacts with KOH. In this manner CO_2 accumulation in the test tube is prevented. Thus the change in air pressure in the test tube measured during respiration results solely from changes in oxygen concentration.

Equipment

- Nova5000
- Three Pressure sensors (150 – 1150 mbar)
- Three 50 ml test tubes
- Three rubber corks
- Three needles, no. 20
- Three latex tubes
- Three three-way valves
- 9 g dry KOH

- Glass beads
- Seeds (pea or bean): 60 dry seeds, 45 swollen seed, 35 germinating seeds

Equipment Setup Procedure

1. Launch MultiLab.
2. Connect the Pressure sensors to Input 1 (I/O-1), Input 2 (I/O-2) and Input 3 (I/O-3) of the Nova5000.
3. Assemble the equipment as illustrated in Figure 1 and Figure 2.
4. A syringe needle (no. 20) is inserted through the cork, until its tip projects slightly out of the cork (Figure 2).

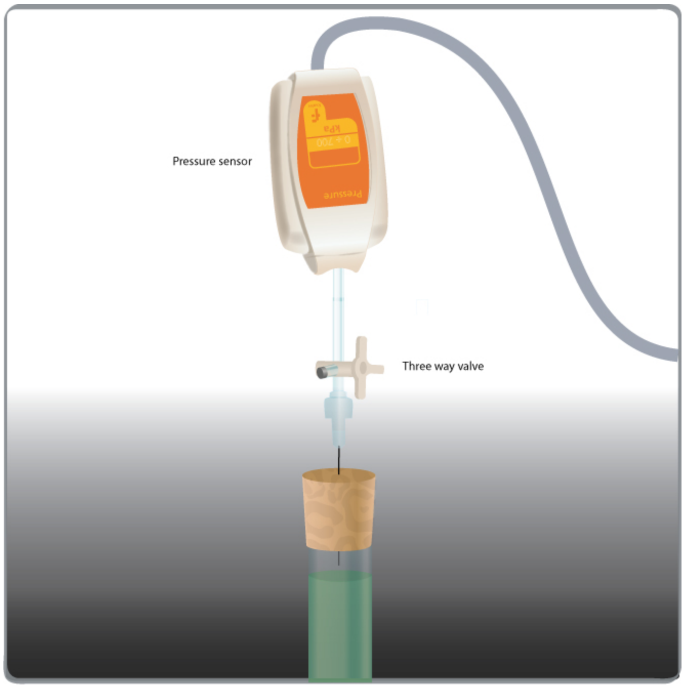


Figure 2

At the other end of the syringe, projecting out of the upper side of the cork, a three-way valve (the one used in infusions) is attached via a very short latex tube (its length must be just enough to hold together the valve and the syringe). A Pressure sensor is connected to the valve through another short latex tube.

Turn the valve until its opening is directed vertically. In this position, air can flow through the valve. In order to stop airflow, turn the valve until its opening reaches a horizontal position.

5. Click **Setup** on the main toolbar and set the data logger up according to the setup specified below.

Data Logger Setup

Sensors:

Input 1: Pressure (150 – 1150 mbar)

Input 2: Pressure (150 – 1150 mbar)

Input 3 Pressure (150 – 1150 mbar)

Rate:

Every second

Samples:

2000 samples

Experimental Procedure

1. Mark the test tubes with numbers, 1-3. Mark a line on each tube 5 cm below its edge.
2. Add 3 g KOH to the bottom of each test tube. Cover it well with glass beads to ensure total separation between KOH and the seeds.
3. Weigh the test tube with the KOH.
4. Add to one test tube dry seeds, to the second - swollen seeds, to the third - germinating seeds, up until the line you previously marked. Count the number of seeds added and weigh the test tubes together with the seeds.
5. Close tightly each test tube with a cork that has a needle inserted in it. Attach to the needle a three-way valve and a Pressure sensor.
6. Click **Run** on the upper toolbar to begin recording data. Follow the pressure recorded on the screen.

Note: *The corks must be efficiently sealed to prevent air leakage in and out of the test tubes.*

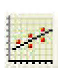
7. Click **Stop**  on the upper toolbar to stop collecting data.

   If the pressure increases after closing the test tubes with the corks, turn the valves until their openings are directed vertically (to enable air flow through them). The pressure in the test tubes should decrease and reach the atmospheric level (about 1000 mbar). To stop airflow through the valves, turn them until their openings reach a horizontal position. Make sure the pressure in the test tubes is maintained at atmospheric level before you start the experiment.

8. After you start the experiment, and as the pressure in the test tubes stabilizes, stop the data logger and start again. Follow changes in the pressure in the test tubes, during the experiment.

9. Save your data by clicking **Save**  on the upper toolbar.

Data Analysis

1. Apply a linear fit to each curve:
   a. Use the First cursor  to select the curve.
   b. Click **Linear fit**  on the main toolbar. The fit equation will be displayed in the information bar at the bottom of the graph window.
   c. The slope of the fit line is the measured rate of pressure changes due to oxygen consumption in the experiment.
   d. The units used are mbar per second.
   e. Multiply the slope 60 times to calculate the change in pressure (mbar) per minute.
2. Compare the slopes received in the three test tubes.
3. Calculate the weight of seeds in each test tube.
4. Calculate the rate of change in pressure per gram of seed weight.

Questions

1. Describe the curves received in the three test tubes. Are they stable throughout the experiment? Are they similar in all test tubes?
2. In which test tube was oxygen consumption the fastest? The slowest?
3. Explain the differences in the rate of oxygen consumption in the different test tubes.
4. Compare the calculated rates per gram weight for each tube. Did it change the relative rates in the different tubes?
5. Explain why we express the change in oxygen consumption in terms of rate per gram weight?
6. What will be the effect of increase in temperature on the rate of oxygen consumption in each test tube?
7. What will be the effect of decrease in temperature on the rate of oxygen consumption in each test tube?
8. What other factors can affect oxygen consumption?
9. Suggest an experimental design similar to the one used in this experiment for how to measure these effects.

Further Suggestions

1. Use an Oxygen sensor to follow the rate of oxygen consumption by seeds during germination.
2. Use seeds of different plants to follow oxygen consumption during germination.
3. Measure the effect of temperature on seed germination.