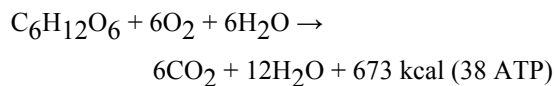


MEASURING RESPIRATION

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INTRODUCTION

The measurement of respiration is very important because it provides a window through which we can determine the metabolic activity of plant tissues. During aerobic respiration, stored food (e.g., carbohydrates, fats, proteins) are combined with oxygen from the atmosphere to produce carbon dioxide, water and the energy needed to maintain the plant cell, tissue and quality of the commodity. The balanced equation for aerobic respiration is shown below.



The rate of respiration is tightly coupled to the rate of cell metabolism because the energy derived from respiration drives all other reactions within a cell. Measurement of respiration affords an easy non-destructive means of monitoring the metabolic and physiological state of the tissues. Nowhere in plant science is knowledge of greater importance than in the area of postharvest physiology, where the events of senescence and ripening are often signaled by abrupt changes in respiratory behavior. For this reason, postharvest physiologists have spent considerable time devising convenient means of measuring respiration.

The rate of any reaction can be determined by measuring the rate at which the substrates disappear or the products appear. Apart from the water produced by respiration, which is relatively trivial compared to the very high water content of plant (and particularly fruit) tissues, all the substrates and products of respiration have been used in determining the rate of respiration.

MEASUREMENTS OF RESPIRATION

A. LOSS OF SUBSTRATE

The respiration of stored commodities can be monitored by measuring the loss of dry matter. In contrast, fresh weight loss is not a good indicator of the rate of respiration. During extended storage periods at warm temperatures the loss of dry matter can be substantial. Water loss is the primary cause of fresh weight loss and it is much more sensitive to changes in relative humidity around the commodity than to the rate of respiration.

All of the heat produced during respiration is lost from the tissue either by conduction to the cold storage room

atmosphere or by vaporization of water in the tissue. If all the energy not captured in ATP (673 - 281 = 392 kcal) were lost from the tissue by vaporizing water, 658 g of water would be lost for each 180 g of glucose oxidized (392 kcal/ 0.596 kcal/g water). Since 108 g of water is produced during respiration, there would be a net loss of 550 g of water. This loss will occur even in a water-saturated atmosphere, and would be much greater than the loss of substrate.

Much easier equation:
 $\text{mg CO}_2 \text{ produced} \times 0.68 = \text{mg sugar consumed}$

When glucose is the substrate, 180 grams of this sugar is lost for each 264 grams of CO₂ produced. The rate of dry weight loss can be estimated as follows:

$$\text{Dry wt loss (g/kg-hr)} = \text{mg CO}_2/\text{kg-hr} \times 10^{-3} \times 68$$

or

$$\% \text{ Dry wt loss (g/100g-hr)} = (\text{mg CO}_2/\text{kg-hr} \times 68 \times 10^{-6})$$

For example: Onions held at 86 °F (30 °C) will produce about 35 mg CO₂/kg-hr. We can use the following equations to estimate the dry weight loss after 1 month of storage.

Much easier to say:
 $35 \text{ mg/kg-hr} \times 0.68 = 23.8 \text{ mg sugar/kg-hr} (= 0.0238 \text{ g})$
 the use of exponents and change in units confuses

$$\text{dry wt loss} = 35 \times 10^{-3} \times 68 = 0.024 \text{ g/kg-h}$$

or

$$\text{dry wt loss} = 0.024 \times 24 \times 30 = 17.2 \text{ g/kg-month}$$

or

$$\% \text{ dry wt loss} = 1.72\% \text{ dry weight/month}$$

In addition to actual weight loss (which could be an important consideration for onions being stored prior to dehydration), the oxidation of substrates can reduce the food value of the commodity (reduced vitamin content), reduced taste quality (ratio and amounts of sugars and organic acids), and accelerate senescence in tissues whose storage reserves have been depleted.

B. CONSUMPTION OF OXYGEN

Oxygen is consumed by respiring tissue, and its loss can be used to measure the rate of aerobic respiration. However, since we work in a sea of oxygen (air is 20.9% oxygen) it is difficult to measure the small changes in oxygen concentration that occur during respiration.

For example, a decrease of 0.3% in oxygen from 20.9% to 20.6% would be difficult to detect because of the large background concentration of oxygen. The change would actually be a 1.4% reduction in the concentration of oxygen. Other problems with using the consumption of oxygen to measure respiration are permeation of oxygen into sampling containers from the air, and the inherent limits of instrument sensitivity.

C. PRODUCTION OF CARBON DIOXIDE

In aerobic respiration one molecule of oxygen is consumed for each molecule of carbon dioxide produced. In the absence of oxygen, i.e. anaerobic respiration, carbon dioxide is produced without the concomitant consumption of equal amounts of oxygen. Under anaerobic conditions, the flow of substrate through the initial steps of respiration (i.e., the anaerobic reactions of glycolysis) is increased to compensate for the loss of energy production. Glycolytic reactions (i.e., fermentation with the production of carbon dioxide and alcohol) extract less than 10% of the energy extracted by aerobic respiration. As a result of the increase use of substrate, the production of carbon dioxide can increase greatly.

A small change in the production of carbon dioxide is easily measured since its concentration in air is around 0.03%. For example, the 0.3% decrease in oxygen during aerobic respiration would be accompanied by a 0.3% increase in carbon dioxide. This 0.3% increase would be easy to detect since it represents a 10-fold increase over the background concentration. Therefore, measurements of the production of carbon dioxide are the preferred methods to measure respiration.

D. PRODUCTION OF ENERGY

The rate of respiration can also be measured as the production of energy (i.e. heat). Substantial amounts of the chemical energy in the substrate appear as heat during respiration. Measurement of the heat of respiration requires sophisticated instruments of calorimetry (i.e. the science of heat measurement). Knowing the rate of heat production is an important factor in designing refrigeration systems for postharvest handling and storage.

Calculation of heat production from the respiration equation shows that production of 1 mg of carbon dioxide yields 2.55 cal. In the language of the refrigeration engineer, a respiration rate of 1 mg CO₂/kg-hr indicates a heat production of 61.2 kcal/metric ton per day (220 BTU per ton of produce per day). The British Thermal Unit is defined as the heat required to raise 1 pound of water by 1 °F.

MEASUREMENT OF GAS PRODUCTION

Almost all practical methods for measuring respiration of plant tissues involve estimating the production of carbon dioxide by the tissue. These methods are generally simple, and have the great advantage of being non-destructive. Indeed they can be used on commodities still attached to the plant. Two general approaches are the static system, and the dynamic system.

Respiration rates are usually expressed as weight or volume of gas produced or consumed per kg fresh weight of product per hour. For example: 125 mg CO₂/kg-h (i.e., per kilogram fresh weight, hour); or 25 ml CO₂/kg-h (i.e. per kilogram fresh weight, hour).

STATIC SYSTEM

In a static system the commodity is placed in a sealed container and the accumulation of carbon dioxide or the depletion of oxygen in the atmosphere is measured over time. This method is employed over brief periods of hours, particularly with smaller commodities or pieces of tissues (meristems, florets, buds).

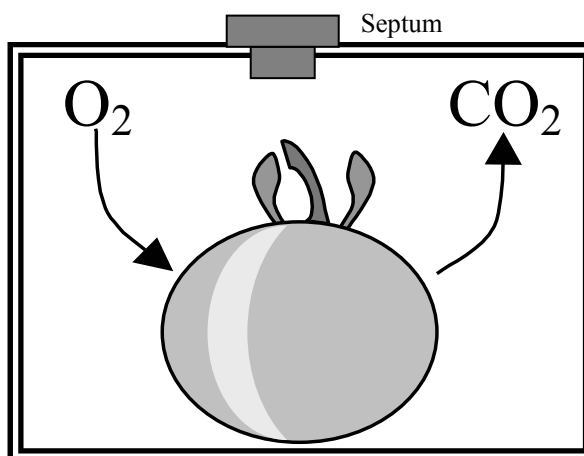


Fig. 1. A static system with a commodity enclosed in a sealed container.

Some respirometers (jargon for a machine that is used to measure respiration) measure the pressure (or volume) change resulting from absorption of respired carbon dioxide. The most notable of these, the Warburg respirometer, was used years ago to determine the pathway of glycolysis, and has driven many postharvest physiologists to strong drink, because of the technical difficulties often encountered in its use.

A static system has the disadvantage of being very susceptible to leaks unless properly sealed. It is also a non-equilibrium system, and the depletion of oxygen, and accumulation of carbon dioxide, or other gases (particu-

larly ethylene) may strongly affect the tissue's rate of respiration. To minimize these problems, the collection time should be as short as needed to accumulate sufficient gas to accurately measure. Note the deviation from linearity in the accumulation of carbon dioxide when the concentration exceeded 0.2% (arrow in Fig. 2).

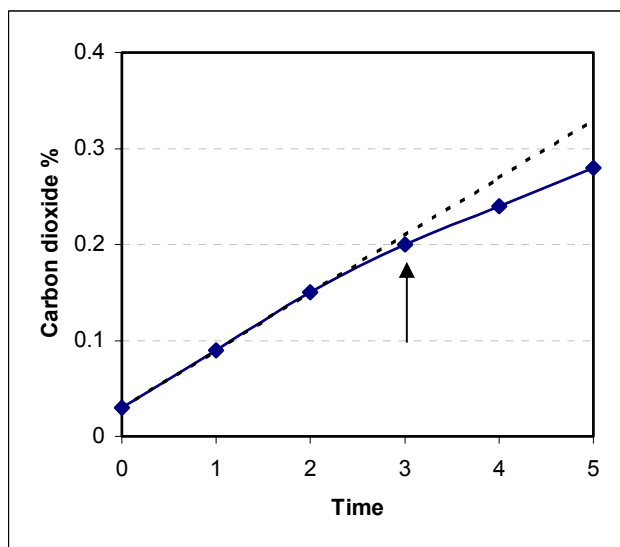


Fig. 2. Accumulation of carbon dioxide over time in a static system.

These problems may also be overcome by including potassium hydroxide (KOH) and potassium permanganate (KmnO_4) in the chamber to absorb carbon dioxide and ethylene, respectively.

To calculate the rate of respiration in a static system you need to know the following:

1. Volume of container
2. Weight of tissue
3. Initial carbon dioxide concentration
4. Length of time
5. Final carbon dioxide concentration.

For example, assume that a 280 g apple is enclosed in a 1560 ml container for 15 min. During that time the carbon dioxide concentration in the jar increased from 0.03% to 0.23%, or the carbon dioxide concentration increased 0.2%. Converting the weight and time units to kg and hr gives a weight of 0.28 kg and a sampling period of $15/60 = 0.25$ hr.

The simplest way to calculate the amount of carbon dioxide produced during the sampling period would be to multiply the difference in carbon dioxide concentrations between the beginning and end of the sampling period (i.e., 0.2%) by the total volume of the container ($0.2\% \times 1560 \text{ ml} = 3.12 \text{ ml}$). Dividing that volume of carbon

dioxide by the weight (in kg) and the sampling period (in hours) would give the rate of CO_2 production.

$$3.12 \text{ ml} / (0.28 \text{ kg} \times 0.25 \text{ hr}) = 44.6 \text{ ml CO}_2/\text{kg hr}$$

This method could be criticized because it does not take into account the fact that the volume occupied by the apple reduced the total gas volume in the jar.

The density of an apple is around 0.8 g/ml, so the volume occupied by the apple would be $280/0.8 = 350 \text{ ml}$. The gas volume in the jar would therefore be reduced to 1210 ml ($1560 - 350 = 1210 \text{ ml}$). Multiplying the gas volume by the increased carbon dioxide concentration and dividing by the weight and sampling duration would then calculate the carbon dioxide production rate.

$$(1210 \times 0.2\%) / (0.28 \text{ kg} \times 0.25 \text{ hr}) =$$

$$2.42 \text{ ml} / 0.07 = 34.6 \text{ ml CO}_2/\text{kg hr}$$

This value is much lower than the previously calculated value because the increase in carbon dioxide occurred in a much smaller volume. Since the volume is smaller, the production of much less carbon dioxide would have been needed to create the same carbon dioxide concentration. Is this variation of the method any better?

We could also assume that 1 g of tissue weight equals 1 ml of volume; then the volume occupied by the apple will be only 280 ml, and not 350 ml. Using this smaller volume for the apple, we get a larger gas volume ($1560 - 280 = 1280 \text{ ml}$), and a larger amount of carbon dioxide produced (2.56 ml vs. 2.42 ml). The production rate is then calculated as

$$(1280 \times 0.2\%) / (0.28 \text{ kg} \times 0.25 \text{ hr}) =$$

$$2.56 \text{ ml} / 0.07 = 36.6 \text{ ml CO}_2 / \text{kg hr}$$

However, we must also be aware that the liquid in the apple fruit will absorb some of the carbon dioxide. The solubility of 100% carbon dioxide in water at 20 °C is 0.878 ml CO_2 / ml water. In an atmosphere containing 0.2% carbon dioxide, the solubility would be 0.00176 ml CO_2 / ml water ($0.878 \times 0.2\%$). If we assume that 1 g of tissue is 1 ml of water, then an additional 0.49 ml of produced CO_2 was absorbed in the tissue ($280 \text{ g} \times 0.00176 \text{ ml/ml}$). The amount of CO_2 produced then becomes:

$$2.56 \text{ ml} + 0.49 \text{ ml} = 3.05 \text{ ml CO}_2$$

and the production rate becomes:

$$3.05 \text{ ml} / 0.07 = 43.6 \text{ ml CO}_2 / \text{kg hr}$$

However, not all the weight of the apple is water. If we assume that 90% of the apple is water the amount of carbon dioxide absorbed by the liquid portion of the apple becomes:

$$2.56 \text{ ml} + (0.49 \text{ ml} \times 0.9) = 3.00 \text{ ml CO}_2$$

and the production rate becomes:

$$3.00 \text{ ml} / 0.07 = 42.9 \text{ ml CO}_2/\text{kg hr}$$

However, the solubility of carbon dioxide into the cell solution is not the same as into pure water. Since we do not know the concentration of the cellular solution, or the solubility of carbon dioxide in aqueous salt and sugar solutions, we must conclude this exercise. The preceding discussion illustrates how the many assumptions made during physiological calculations can significantly alter the answer.

We can conclude that under 'normal' physiological conditions, the rate of carbon dioxide production can be calculated to within about 5% of its 'true' value by ignoring the volume changes introduced by the tissue. The volume of carbon dioxide produced is therefore calculated by multiplying the change in concentration times the entire void volume of the container.

DYNAMIC SYSTEM

For long-term experiment, respiration is often determined in a system where the commodity is placed in a sealed container ventilated with a known rate of flow of air. The respiration (either oxygen uptake or carbon dioxide production) is readily calculated from the measured concentration difference between the inlet and outlet of the container and the known flow rate.

The concentration of carbon dioxide in the flow leaving the container increases until as much carbon dioxide leaves the container (concentration x flow) as is produced by the tissue (arrow in Fig. 4). At that time the system is in equilibrium and gas samples can be taken to analyze for carbon dioxide concentration.

To calculate the rate of respiration in a dynamic system you need to know the following:

1. Weight of tissue
2. Inlet carbon dioxide concentration
3. Flow of gas
4. Outlet carbon dioxide concentration.

This system has the advantages that leaks are not critical and that atmospheres of various compositions can be used to ventilate the container. Also, undesirable changes in gas concentrations are minimized by the continued replacement of the atmosphere.

Production of carbon dioxide or ethylene, or consumption of oxygen is calculated by multiplying the increase (or decrease) in concentration between the inlet and outlet tubes times the flow rate. Dividing by the fresh weight (in kg) of the tissue gives the rate of carbon dioxide production in ml per kg hr.

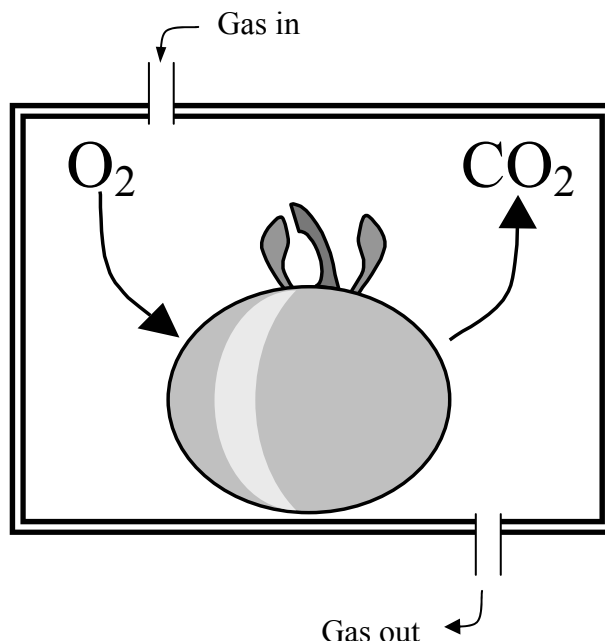


Fig. 3. A dynamic or flow-through system with a commodity enclosed in a container through which gas is flowing.

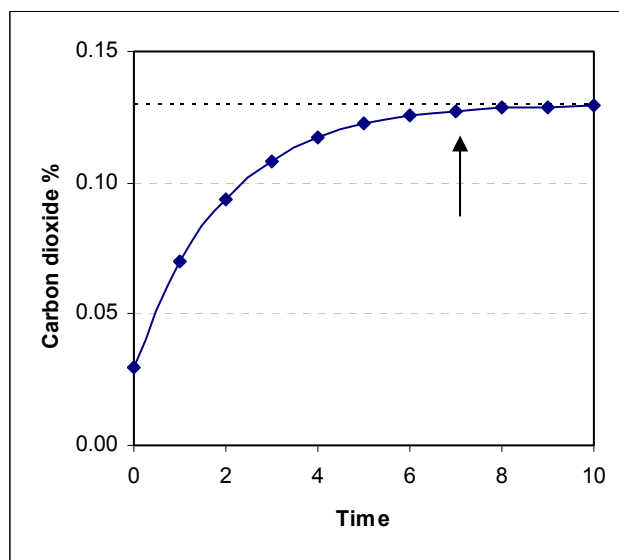


Fig. 4. Accumulation of carbon dioxide over time in a dynamic system.

For example, assume that a 280 g apple is enclosed in an 843 ml jar. The carbon dioxide concentration increases from 0.03% to 0.23% as air flows through the jar at 100 ml/min. It usually takes three volume changes for a flow-through system to come into equilibrium, so it will take

$$3 \times 843 \text{ ml} / 100 \text{ ml/min} = 25.3 \text{ min}$$

for this system, to come into equilibrium. The concentration of carbon dioxide increases 0.20%, so the amount of carbon dioxide produced per hour is

$$0.2\% \times 100 \text{ ml/min} \times 60 \text{ min/hr} = 12.0 \text{ ml CO}_2 \text{ hr}$$

Dividing by the 0.28 kg fresh weight gives

$$(12.0 \text{ ml CO}_2/\text{hr}) / 0.28 \text{ kg} = 42.8 \text{ ml CO}_2/\text{kg hr}$$

The accuracy of the dynamic method is dependent of knowing the exact flow rate. The container volume and the flow rate are both important in determining the time it will take for the system to come to equilibrium, but as long as the system is at equilibrium, the volume of the container is not relevant to the calculations. Small leaks are also not important as long as the gases are thoroughly mixed within the container.

SAMPLING AND ANALYZING

Gas samples can be taken with gas-tight plastic syringes by inserting the syringe needle through a septum in the container walls or into flexible tubing protruding from the container. The material used to make most of the glass and plastic sampling syringes is very impermeable to carbon dioxide and oxygen, so samples can be held in these containers for days without significant contamination by gases in the surrounding atmosphere. The samples are injected in an analyzer and the output compared to standards to arrive at the correct gas concentration in the sample.

Qualitative and quantitative measures of the component gases in the sample can be done with a variety of methods and instruments. Early methods of gas analysis relied on changes in the volume of the gas sample when specific gases were absorbed. These methods are of only historical interest today. However, some of the early colorimetric methods are still used. The reaction of a specific gas with a reagent immobilized on a solid support causes a color change that identifies the gas and can be used in certain configurations to estimate the concentration of the gas.

Modern methods of gas analyses rely of electronic instruments. Some instruments are designed to measure a specific gas. For example, the infrared analyzer is specific for carbon dioxide, and the paramagnetic or electrochemical analyzer is specific for oxygen. These instruments do not require the separation of a gas sample into its individual gas components.

The gas chromatograph separates a gas sample into its individual components by passing the sample down a long tube filled with a coated solid support. The sample is injected into a stream of inert gas (usually nitrogen) that carries it down the tube. The coating and support

are selected to achieve the desired separation of the gases. Upon emerging from the tube, the separated gases pass through a detector that measured some property of the gas. Thermal conductivity detectors measure the capacity of the sample to remove energy from a heated filament. Ionization detectors measure the electrical conductivity of the gas after it has been ionized by combustion (flame ionization detector) or irradiation (photo ionization detector).

Samples need not be drawn if the respired gases can be collected. In a dynamic, or flow-through system the commodity is placed in a sealed container through which a stream of gas is passed. The exit stream is passed through a column containing caustic solutions or granules that absorb the respired carbon dioxide. Respiration is determined by subsequent titrimetric or gravimetric analysis of the material in the column. This system is relatively easy to set up, and is independent of air flow rates, but any leaks in the tubes leading from the respiration container (which is usually impossible to seal completely!) causes erroneous results. This method is rarely used because large amounts of plant tissue and long sampling times are required, and the method is not as accurate as the other instrumental methods described previously.