Carbon Dioxide Effects on Fruit Respiration [1]

I. Measurement of Oxygen Uptake in Continuous Gas Flow

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Fruit physiologists have been concerned with carbon dioxide as a product of the respiratory process and as a factor in the environment surrounding the fruit. Measurement of CO_2 evolution was favored over determinations of oxygen uptake because of the simplicity of the determination, but a number of investigators realized that CO_2 may be produced by anaerobic as well as aerobic pathways of metabolism. Therefore, carbon dioxide is not a sufficient index for establishing the nature of the biological oxidation in the material under study. This realization is of particular significance when fruits are subjected to atmospheric conditions that differ materially from those in air.

The interest in modified atmospheres was prompted by the desirability of prolonging storage life, and the chief emphasis was on the observation of fruit quality in increased carbon dioxide and reduced oxygen in the storage environment as compared to ordinary air. Few investigators have concentrated on a systematic change in one component while maintaining constancy in the other. Still fewer have determined respiration as a function of CO_2 tension, since as long as CO_2 measurements were employed by conventional techniques it was not feasible to determine the small amount of carbon dioxide added to the atmosphere containing a relatively high concentration of carbon dioxide. Attempts to develop infrared analysis to achieve such determinations proved excessively complicated. Lack of suitable analytical methods had limited the use of oxygen consumption as a measure of respiration in intact tissues, until Pauling, Wood, and Sturdivant (3) took advantage of the fact that oxygen has an unusually high paramagnetic susceptibility while most other gases are only slightly diamagnetic. They devised a simple and sensitive means of measuring this property of gases and built an instrument which would detect very small changes in the partial pressure of oxygen.

The paramagnetic principle was used by the A. O. Beckman Co. to develop a commercial oxygen analyzer. Since their null type instrument was capable of determining changes in oxygen concentration as small as 0.02 %, it seemed suited to the measurement of respiratory activity in atmospheres high in carbon dioxide. This instrument is characterized by excellent sensitivity, freedom from interference, and linearity over the required range. It also has the unique advantages of requiring no specially calibrated gas mixtures for standardization and of being easily adaptable for automatic sampling and recording. Since the gas is not altered by the determination it may be trapped for other analyses. On the other hand, it has the disadvantage, also inherent in most other methods, that the flow rate must be determined precisely. Several years ago the A. O. Beckman Co. (Process Instrument Div., Beckman Instrument Co., Fullerton, Cal.) agreed to build an instrument designed to automatically sample and record respiratory activity of fruit subjected to gas mixtures high in CO_2 and containing from one to 25 % O_2.

► Principle of the Oxygen Analyzer. A schematic illustration of an oxygen analyzer is shown in figure 1. This diagram was supplied by the Beckman Co. and depicts a simplified instrument which illustrates the principle of the analyzer. The paramagnetic detector is shown in the left section of the diagram and consists of a dumbbell-shaped test body suspended on a quartz fiber in the non-uniform field of a permanent magnet. The test body is free to rotate on the fiber in response to magnetic and electrostatic forces.

The test body itself is paramagnetic and in the absence of a paramagnetic gas tends to orient in the position of maximum magnetic flux. As oxygen is admitted to the unit, the test body tends to rotate out of the position of maximum magnetic flux in response to the gas becoming more paramagnetic. The degree of rotation is proportional to the difference between the volume magnetic susceptibilities of the test body and the gas that it displaces.

A mirror attached to the quartz fiber just above the dumbbell-shaped test body reflects a beam of light to the apex of a front silvered prism, which divides and directs the reflected light beam to two photocells in proportion to the angle of deflection of the test body. Thus any deflection from the null position of the test body causes an unbalance of the output of the two photocells which results in movement of the recorder pen. A precision potentiometer,

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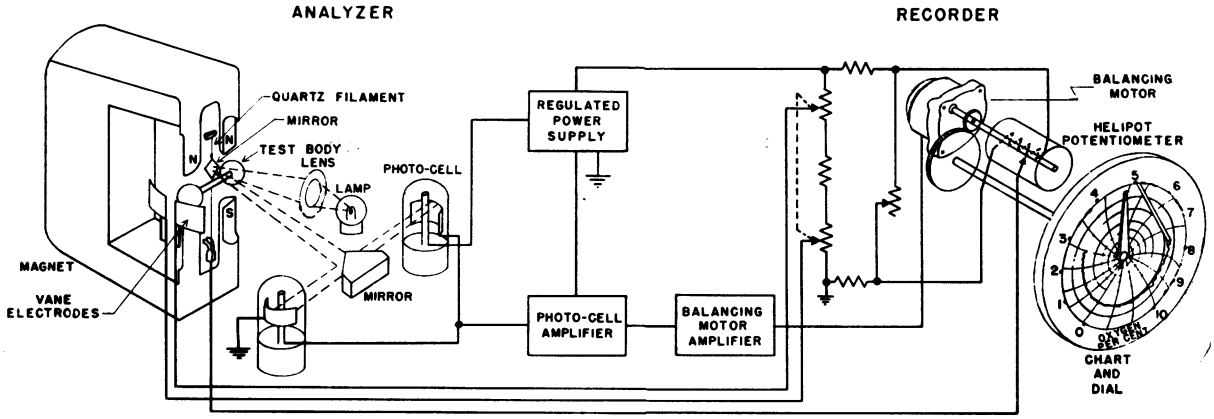

Fig. 1. Schematic diagram of oxygen analyzer.

attached to the pen-drive mechanism of the recorder, changes the potential applied to the gold-plated test body. This variable potential and the fixed potential difference applied to the two vane electrodes located symmetrically with respect to the angle of displacement of the test body, constitutes a heterostatic electrometer which holds the test body in its null position. Thus any change in magnetic force due to a change in the partial pressure of oxygen is balanced by an applied electrostatic force which is proportional to the voltage developed by the potentiometer. Accordingly, changes in oxygen concentration are linearly related to voltage changes recorded on the chart.

Movement of the test body can be achieved by two independent means: the potential applied to the test body itself, or to the vane electrodes. With nitrogen in the analyzer the recorder is on zero, the feedback to the suspension is at its lower limit, and the suspension is brought to null position by adjustment of the mechanically coupled potentiometers attached to the vane electrodes. When air is passed through the detector, the pen is adjusted to 21 % oxygen on the chart by adjustment of the span potentiometer which regulates the feedback potential to the suspension. This permits standardization of the instrument through the use of only oxygen-free nitrogen and air. The scale is linear and no other gas mixtures are required for calibration on the instrument.

For most measurements of respiratory activity it is necessary to determine the decrease in oxygen concentration of about 0.3 to 1.5 % O_2 with a precision of at least 0.02 % O_2. When respiratory activity is to be measured in an atmosphere of air, conventional oxygen analyzers with a span of 19 to 21 % or any of the conventional CO_2 methods can be used. However, we wished to be able to measure the oxygen uptake of a series of samples subjected to gas atmospheres in which the percentage of oxygen and carbon dioxide supplied to the sample could be at any level between 1 and 25 % oxygen.

For this purpose the oxygen analyzer was equipped with the multisampling system, a timing device, sequence switch, an additional pair of motorized potentiometers, and span adjusting components. With this equipment the instrument can automatically sample gas streams leading to and from a series of jars of fruit in any particular sequence. It determines the percentage of oxygen in the gas stream leading to a sample on a 25 % scale and then finds the difference in the percentage oxygen in the gas streams leading to and from a sample on a 2 % scale. On this expanded scale differences of 0.02 % oxygen can be detected irrespective of the concentration of oxygen being supplied to the sample.

Prior to the discussion of the detailed operation of the instrument it will be useful to describe a typical gas mixing and metering system used in this laboratory.

► Preparation of Gas Mixtures. Many of the experiments to be discussed required the use of gas mixtures high in carbon dioxide and low in oxygen. Since commercial gas mixtures were of unreliable composition, gas mixtures are made in the laboratory by means of a combination of capillary type flowmeters. In figure 2 is illustrated a typical flowmeter and mixing board. Air from the laboratory supply line is reduced to 15 pounds per square inch and then regulated to a pressure equivalent to approximately three feet of water by means of a barostat. Nitrogen and oxygen are supplied from 220 ft^3 high pressure cylinders and the pressure is reduced to 3 lb per square inch by means of two stage pressure reduction valves. Carbon dioxide is supplied from dry ice put in a CO_2 converter. Liquid CO_2 supplied in high pressure cylinders (from refinery sources) has been found unsuitable for these experiments due to the occasional contamination by a trace of a gas, tentatively identified as carbon monoxide, in sufficient concentration to show a physiological effect on fruits. Gas streams supplied to the mixing board are controlled by means of precision type metering needle valves. Flow rates of three gases to be mixed are determined

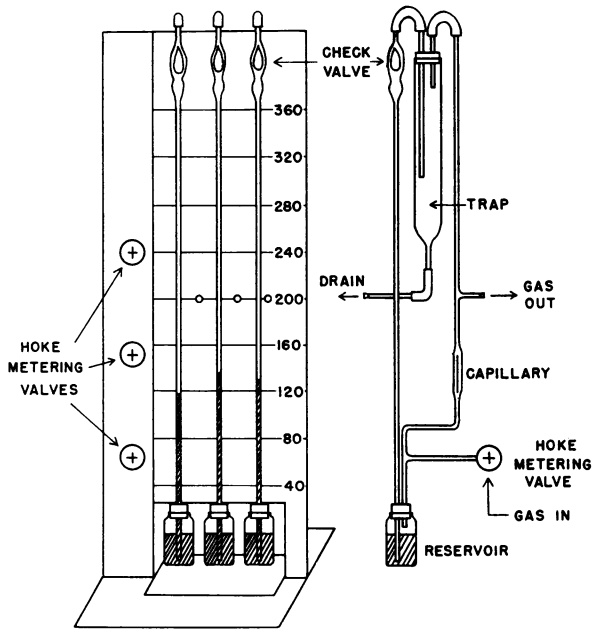

Fig. 2. Front and side views of gas mixing board.

by means of three capillary type flowmeters in parallel. Of many types of flowmeters tried in this laboratory, the single arm flowmeter (fig 2) has proven the most trouble-free and is simple and inexpensive to build. Manometer tubes are 5 mm O.D. tubing and the reservoir is a 2 oz wide mouth bottle. The check valve is installed in the manometer tube to prevent blowing over of the manometric fluid due to an accidental overadjustment of the flow rate. An additional precaution against clogging of the capillaries is provided by a trap to catch the manometric fluid in case the check valves stick. Capillaries are 2 inches long of precision bore capillary tubing supplied by the Fischer & Porter Co., Hatboro, Pa. Bores are of sufficient diameter to give a deflection of 100 to 360 mm for the flow desired, and are calibrated by use of a wet test meter or some other standard method (2). The manometric fluid used is a 1 % aqueous solution of sodium taurocholeate colored with Evans blue. It is convenient to mount approximately 20 flowmeters on a single panel and then combine the appropriate outlets to make the gas mixtures.

► Gas Handling System. As mentioned before, the analyzer acts as a difference analyzer and, therefore, the gas stream must be divided, part going directly to the analyzer and the remainder being passed over the sample of fruit and thence to the analyzer. Figure 3 is a flow diagram which shows how standardizing gas and two samples of fruit are arranged.

Air must always pass through the first pair of ports of the multisampling system to provide a trace on the recorder chart for the case where there is no difference in the oxygen concentration between two gas lines. For the samples of fruit, gas is taken from a mixing board (fig 3), humidified, and, if the effect of ethylene is to be shown, divided by a T joint and passed to flowmeters F1 and F2. The division of flow can be controlled by a screw clamp placed adjacent to F1 or F2.

Ethylene is introduced just beyond F2. The concentration of ethylene normally desired for fruit respiration experiments lies between 0.05 and 1,000 ppm. It is impractical to prepare these dilute mixtures directly from pure ethylene due to the difficulty of maintaining flow rates less than 1 ml/min at a constant value. To circumvent this difficulty, ethylene-air mixtures 10 to 100 times the desired concentration are made up in high pressure gas cylinders. A cylinder is evacuated and an appropriate volume of pure ethylene drawn into the cylinder from a gas pipette. The cylinder is then attached to a high-pressure laboratory air line and pumped up to 100 pounds per square inch. For example, for a cylinder normally used for gases at 2,000 lb pressure rated at 220 ft^3, the addition of 1.4 ml of pure ethylene and then air to 100 lb per square inch gives a mixture of approximately 5 ppm. If 3.5 ml/min of the 5 ppm ethylene mixture is then introduced via flowmeter F5 and 350 ml per minute of air or a gas mixture introduced via flowmeter F2, the concentration of ethylene in the final mixture will be 0.05 ppm. If 35 ml of the 5 ppm mixture is introduced at flowmeter F5 the final concentration will be 0.5 ppm. These flow rates are easily measured by conventional capillary flowmeters and the contents of a single cylinder will last for several days when used at these flow rates. A similar dilution of other concentrations can be used to obtain ethylene concentrations up to 1,000 ppm. Mixtures containing more than 10,000 ppm or 1 % ethylene should not be used because such concentrations approach the explosive limit of 3 % ethylene in air. The bubble counter after flowmeter F5 is used as a check on the proper function of this flowmeter, since water will occasionally condense in the capillary and give false readings at very low flow rates.

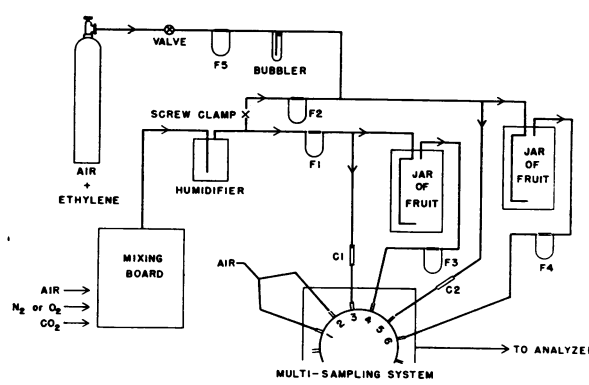

Fig. 3. Flow diagram for modified air experiment using a multi-sampling system (see text).

The gas streams from F1 and F2 are next divided to approximately 100 to 150 ml going directly to the multi-sampling system ports 3 and 5 (fig 3) and 200 ml per minute going to the jars of fruit and thence to flowmeters F3 and F4 and ports 4 and 6 of the multi-sampling system. The flow rates to ports 3 and 5 need not be accurately known. The gas stream is divided by inserting capillaries in the lines at C1 and C2 which are slightly smaller than the capillaries in flowmeters F3 and F4. The flow rates through flowmeters F3 and F4 are adjusted to 200 ml per minute or any other suitable flow rate between 100 and 500 ml per minute by means of pinch clamps on the tubing adjacent to flowmeters F3 and F4. This range is optimal for the operation of the analyzer. A flow of 100 ml/min is required to achieve complete change of sample in the analysis cell within the time allowed. With rates higher than 500 ml/min the pressure in the analyzer cell is likely to increase above atmospheric.

► Operation of Analyzer. The analyzer consists of four parts shown in figure 4: A, the multi-sampling system, B, the detector, C, a timer, sequence switch and associated potentiometers, and D, a potentiometric recorder. Figure 4 is a considerably simplified diagram and is provided only to illustrate the operation of the analyzer.

Samples to be analyzed are selected by the multi-sampling system shown in detail in figure 5 and in relation to other components in figures 3 and 4. All gas lines are connected to ports located on a fixed circular rim or turret as illustrated in figure 3. A central disk (see fig 5) carrying a take-off tube to the analyzer through a gas-tight universal joint rotates at intervals of 7 or 8 minutes from one port to the next, connecting the take-off tube with each port in the series. A timer starts the motion of the disc and indexing screws on the rim of the disc actuate a micro-switch which stops the take-off tube at the appropriate port. The system has 50 ports and indexing screws for each port. These screws may be removed easily so that any number or any combination of the 50 ports can be selected.

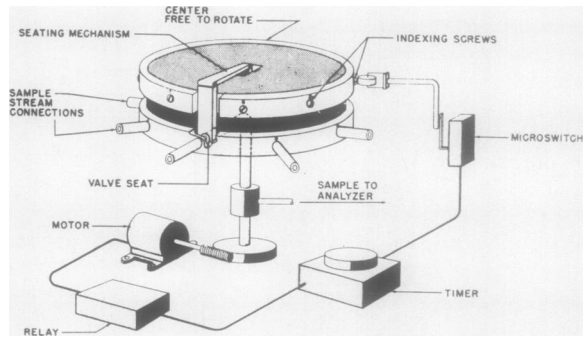

Fig. 5. Expanded diagram of multi-sampling system.

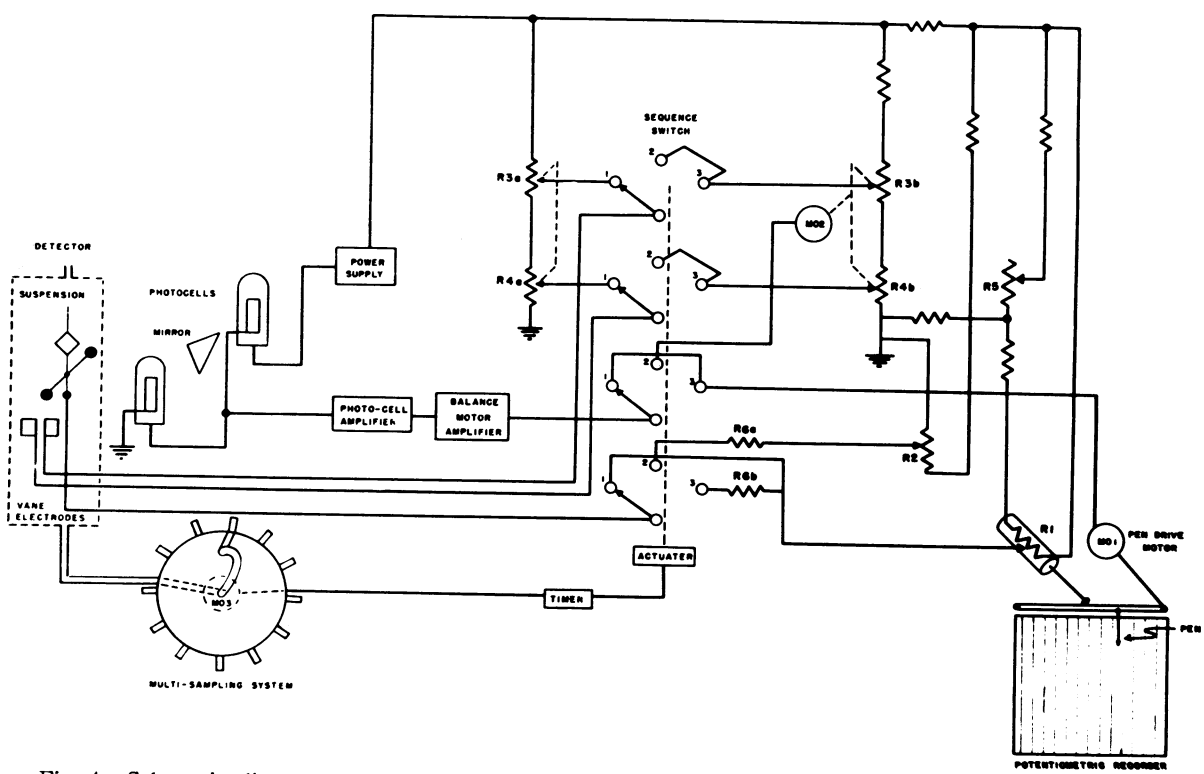

Fig. 4. Schematic diagram of automatic oxygen analyzer.

The analyzer is set up to operate as a difference analyzer, that is, it determines the difference in the percentage of oxygen between two gas streams. Therefore, a pair of ports must be used for each analysis as is shown in figure 3. A reference gas, normally air, must be passed through the first pair of ports, leaving one to 24 pairs of ports available for use.

The analysis of each sample lasts 15 minutes and consists of three steps, of which the first two require 4 minutes each and the last, 7 minutes. For the first step, the multi-sampling system is on an odd-numbered port sampling the inlet gas stream. The sequence switch is on position one (fig 4), and the recorder is operating on a scale such that 25 % oxygen equals full scale. The suspension is brought to its null position by feedback from R1 coupled to the recorder pen drive. When air is supplied to the detector the recorder trace occurs at division 84, which is equal to 21 % oxygen on the chart of 100 divisions (trace 1 of fig 6). The 4 minutes allowed for this step permit a complete sample change of the 16 ml volume of the detector if the flow rate is approximately 100 ml/min.

Referring again to figure 4, step 2 is started by the timer causing the actuator to advance the sequence switch to position 2. The multi-sampling system does not advance, but remains on the inlet gas stream. The sensitivity is multiplied by 12.5 by virtue of R6a being added to the circuit so that the entire width of the chart equals 2 % oxygen. Now R1 is disconnected and the potential on the suspension is supplied by the manually adjusted potentiometer R2. The suspension must now be out of null position and is restored to null by reduction of the voltages on the

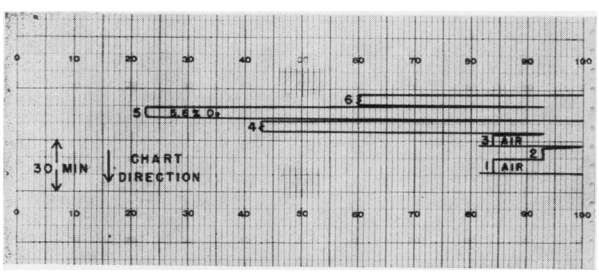

Fig. 6. Strip chart record of 2 % reference standardization and analysis of one sample under air and another under 5 % O_2 and 10 % CO_2 (see text).

vane electrodes by the motorized potentiometers R3b and R4b. The recorder continues to make its trace at the same chart division as in step 1, but is actually disconnected during step 2, as can be noted by the position of deck 2 of the sequence switch. Trace 1 of figure 6 represents the 8 minutes of steps 1 and 2.

As the sensitivity of the instrument is now multiplied so that the entire width of the chart represents 2 % oxygen, the function of step 2 is to adjust electrically the detector to the 2 % scale with the inlet gas in the analysis cell by reduction of the potential on the vane electrodes. R2 is so set that with the inlet gas stream in the detector the pen of the recorder will trace near the right-hand margin of the chart, irrespective of whether the inlet gas contains 2 or 25 % oxygen or any value in between these limits.

For step 3, the sequence switch advances to position 3 and the multi-sampling system advances to the next port. Now motor 2 (Mo 2 in fig 4) is disconnected and the voltage on the vane electrodes is fixed as in step 2 while the null position of the detector is adjusted by R1 coupled to the recorder. The span is still 2 % by virtue of R6b being in the circuit. If the oxygen concentration is the same during this step as in steps 1 and 2, the recorder will trace near the right-hand margin of the chart as shown in trace 2 at division 93 in figure 6. Indeed, the first two ports must always carry the same gas, generally air, to establish this 2 % scale reference point.

The next cycle is started by the sequence switch returning to position 1 and the valve advancing to position 3. For the portion of the chart shown in figure 6, trace 3 represents this cycle and air was being fed to the valve. The recorder again records at division 84 or 21 % oxygen during steps 1 and 2 as represented by trace 3 on figure 6. At step 3 the valve advances to port 4, which is accepting air that has passed over a sample of fruit. The recorder now moves downscale to division 43 to make trace 4. On this scale each division equals 0.02 % oxygen; therefore the difference between 93, the 2 % reference point, and 43 multiplied by 0.02 equals 1.00 % oxygen used up by the fruit.

For the next cycle shown on the trace in figure 6, 5.6 % oxygen was supplied to port 5 of the valve and to a sample of fruit. For this position the recorder moves downscale to division 22.5, representing 5.6 % oxygen on the 25 % scale. When the instrument switched to position 3, the pen moved to the 2 % reference line, that is to division 93. As gas from port 6 entered the detector cell, the pen moved immediately back to division 60 to draw trace 6. This sample of fruit used 0.66 % O_2 [(93–60) × 0.02]. The end of each cycle is represented by the pen moving to division 100.

Respiration rates are generally reported in terms of ml O_2/kg/hr at standard conditions according to the formula:

$$\text{Rate} = \frac{(\%\ O_2)\ (\text{Flow in ml/hr})\ (273)\ (P)}{(\text{Weight in kg})\ (T)\ (760)}$$

Pressure and temperature are determined at the point where flow is measured.

Experimental Data

The instrument was checked for accuracy of values obtained, linearity of response, and accuracy of 2 % spans at different oxygen levels by comparison with Orsat analysis using chromous chloride (4) as the oxygen absorbent and a tapered gas burette so

Table I

Comparison of Oxygen Concentrations by Paramagnetic and Orsat Analyses

Trial	Orsat Analysis % Oxygen			Oxygen Analyzer Scale division 2 % Scale			
	Mixture 1	Mixture 2	Diff.	Mixture 1	Mixture 2	Diff.	% Oxygen
A	3.17	2.26	0.91	95	49½	45½	0.91
B	15.60	13.90	1.70	89	4	85	1.70
C	4.60	3.00	1.60	91½	10	81½	1.63

that high accuracy could be obtained at low oxygen concentrations. With the burette used, accurate determinations could not be made by Orsat analysis at oxygen levels above 16 %. Data shown in table I indicate satisfactory agreement with Orsat analysis when the concentration of oxygen being introduced varied from 3.17 % to 15.50 %, and where the differences in oxygen concentration between odd and even numbered ports varied between 0.91 and 1.70 % oxygen.

Table II

Reproducibility of 2 % Reference Points Using Various Gas Mixtures

Cycle	Port	1 & 2	3 & 4	5 & 6
	Gas	Air	5 % O_2 95 % N_2	5 % O_2 10 % CO_2 85 % N_2
1		71	70½	71½
2		70½	71	70
3		70½	71½	70
4		70	70	70½
5		70½	69½	70½
6		70½	...	70½
Avg.		70½	70½	70½

Numbers refer to the chart division. One division = 0.02 % oxygen.

Reproducibility of the 2 % reference line when gas mixtures of different oxygen and carbon dioxide concentrations are passed through the instrument is shown in table II. In this case a typical experimental setup was made except that no fruit was put in the jars, so that gas entering the odd and even numbered ports was identical. The 2 % reference point was adjusted to division 70½ so that if deviations did occur they would not go off scale. Data of table II show that several gas mixtures gave a 2 % reference line reproducible within 0.02 % oxygen. This has been repeated with a wide variety of other gas mixtures. This type of check is made prior to every experiment because any small leak in the system will cause serious error, especially when gas mixtures low in oxygen are used.

Discussion

The greatest advantage obtained through the use of an oxygen analyzer for measurement of respiration or photosynthesis is that the determination can be made in any concentration of carbon dioxide and at any oxygen level between one and 25 % with an accuracy adequate for experiments on intact organs. The zero point of the instrument is adjusted by passing any oxygen free gas and the span set by passing air so that no specially prepared gases are required for standardization. The scale is linear and no corrections need be applied or calibration curves established. The instrument is easily adaptable to automatic sampling and recording.

The automatic sampling and recording features are of particular advantage where respiratory or photosynthetic changes occur rapidly. A single sample can be attached to the valve and a continuous record of oxygen utilization or production obtained. In figure 7 is shown a curve of the respiratory activity of a culture of *Penicillium digitatum* Sacc. which il-

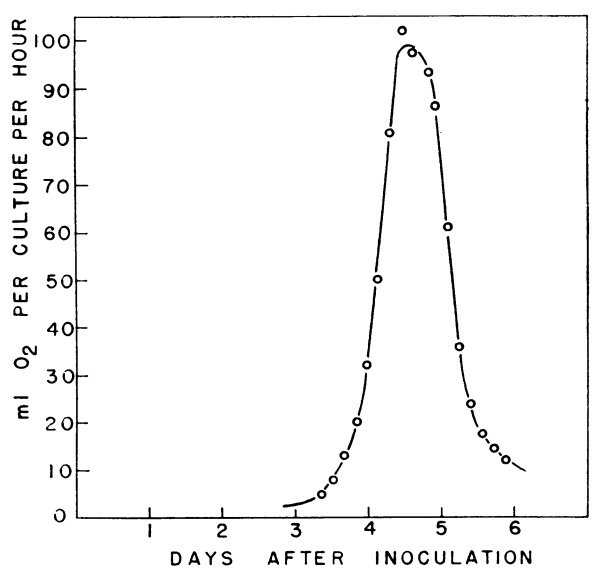

Fig. 7. Oxygen uptake of a culture of *Penicillium digitatum*.

lustrates the value of frequent and automatic recording. There was essentially no oxygen uptake for 3½ days, followed by a rapid rise and decline in activity during the next 48 hours. The major change in respiration of this culture was confined to an 18 hour period. Another example of the value of frequent determinations is found in the case where enzymatic activities of fruit are to be determined at various stages of the climacteric rise in respiration. Here frequent determinations of respiratory activity of a number of individual fruits permits the choice of fruit for analysis which are at precisely known stages of the climacteric.

The instrument can be used equally well for measurement of photosynthesis. We have determined photosynthetic activity of intact tobacco plants, detached leaves, and barley seedlings. Calvin has used a similar instrument for determination of photosynthesis of Chlorella (1).

Summary

The determination of respiratory activity of fruits and tissues by a paramagnetic oxygen analyzer is described. The primary advantage of the method is that respiratory activity can be determined in the presence of added carbon dioxide and at any oxygen level from 1 to 25%. No specially prepared gas mixtures are required for calibration and the instrument is easily adapted to automatic sampling and recording. The oxygen analyzer is particularly useful where rapid changes in rate are to be followed or for measuring photosynthetic activity. Gas handling systems used in conjunction with the oxygen analyzer are described.

Acknowledgment

Figures 1 and 5 were supplied by the Beckman Instrument Co.

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