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An Apparatus for the Automatic Measurement of Oxygen Uptake by Electrolytic Replacement of the Oxygen Consumed

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In studies of the respiration of plant tissue, particularly in relation to terminal oxidases, measurement of the rate of uptake of oxygen from an atmosphere of controlled oxygen pressure is necessary. For short periods of time and small amounts of tissue a Warburg type of apparatus is suitable but in studies of intact tissue, where whole organs must be used (0.5–200 g.) and where the duration of an experiment is prolonged, an apparatus that maintains the partial pressure of oxygen constant while giving a continuous record of uptake is needed. The apparatus to be described replaces the oxygen consumed by the tissue by oxygen generated electrolytically, and automatically records the volume added. This principle of electrolytic replacement of oxygen has been used before (Fernandes, 1923; Wetzel, 1927; Bennet-Clark, 1932; Ulrich, 1940), but the accuracy of the estimations has been greatly increased by the use of a new type of electrolytic oxygen generator based on the use of palladium and by a new method of measuring the oxygen added. The apparatus was designed to measure the oxygen uptake by 5–10 g. of peas and has been extensively used in conjunction with an automatic method for measuring the carbon dioxide produced (Wager, 1957).

Recently it has been used to determine the oxygen uptake by 100–150 g. of potatoes from atmospheres of different oxygen content (Mapson & Burton, 1962).

METHODS

Principle of the method (Fig. 1)

The tissue is enclosed in an airtight respiration chamber above a solution of sodium hydroxide, which serves to absorb the carbon dioxide given out. This chamber is connected to a compensating volume through a narrow U-tube manometer and also to an electrolytic oxygen generator (Figs. 1 and 3). A platinum electrode is fixed

Fig. 1. Block diagram of the apparatus.

just above the meniscus in one limb of the manometer and as the tissue absorbs oxygen the meniscus rises and finally touches the electrode. At regular intervals the manometer is tested for electrical continuity by the 'timer' and when it occurs the relay system switches a constant current through the oxygen generator for a standard time. The 'pulse' of oxygen so produced blows the meniscus clear of the electrode. Since each pulse of oxygen is of equal volume the rate of respiration is calculated from their frequency as recorded by the chronograph.

During a run there is no significant change in partial pressure of oxygen in the respiration chamber (pulse volume : chamber volume is 1:6000), and the apparatus therefore can measure oxygen uptake from gas mixtures containing any partial pressure of oxygen up to 1 atm.

Description of the apparatus

Oxygen generator. The basic requirements of the oxygen generator are that it must produce pure oxygen in exact equivalence to the current passed, and it must be sealed from the atmosphere. The electrolysis of alkaline copper sulphate was found to produce some ozone and to be liable to side reactions. A satisfactory alternative is to electrolyse a solution of sodium hydroxide by using a large palladium cathode which absorbs the hydrogen produced. All parts of the cathode must receive an equal current, the amount of palladium must be sufficient to absorb all the hydrogen as it is produced (this is considerably less than the total hydrogen capacity) and the movement of oxygenated electrolyte to the cathode must be restricted. These requirements are met by the generator shown in longitudinal section in Fig. 2. It consists of two 35 mm. flat

glass joints separated by and clamped to a polystyrene plate through which passes a 2-3 mm. bore glass tube sealed with a sintered-glass disk. The cathode consists of 25 g. of 1 mm. diam. palladium wire wound into a concave spiral and bedded down on a polythene mount. The concavity is such that all parts of the palladium spiral are approximately equidistant from the exit to the anode compartment and hence receive equal current. (The spiral is held together by 'spokes' of palladium wire on the outer side.) This cathode absorbs reliably about 200 ml. of hydrogen and the generator therefore can deliver up to 100 ml. of oxygen to the respiration chamber. The hydrogen is discharged from the palladium by disconnecting the generator from the respiration chamber and passing an electric current through it in the reverse direction until the original cathode just evolves oxygen. The anode consists of 2-3 cm. of 1 mm. diam. palladium wire, which in use rapidly becomes black.

These generators do not appear to produce ozone mixed with the oxygen, probably because the oxygen is formed on a blacked palladium electrode.

Two similar generators are used in series, one connected to the respiration chamber and one to a gas burette so that a running check can be kept on the oxygen generated and hence of the size of each pulse of oxygen.

Respiration chamber and manometer. The respiration chamber is rigidly constructed of glass and polythene of a shape suitable to the tissue to be used and with a lid, which, after sealing, can be clamped to the base. This chamber, the manometer bridge, the compensating volume and the oxygen generator are made with all-glass connexions and mounted rigidly on a small stand (Fig. 3).

A volume change of less than 0.1 ml. in 600 ml. causes the electrode in the manometer to make or break contact with

Fig. 2. Diagrammatic longitudinal section of the electrolytic oxygen generator.

the meniscus. To obtain this sensitivity one limb of the manometer is constricted to an internal diameter of 4 mm. and the electrode consists of 5 mm. of 0.004 mm. diam. platinum wire welded to a basal portion of about 1 mm. diam. platinum wire.

For satisfactory operation the manometer fluid must wet the glass, have a low surface tension and density and a reasonably good electrical conductance; a solution of 1% sodium carbonate in 50% (v/v) ethanol-water is suitable.

Electrical circuits (Fig. 4). The electrical circuits have to switch on the electrolytic oxygen generator for a standard time when the electrode in the manometer makes contact with the meniscus, to record the time at which this occurs and to operate with the small current that it is practicable to pass through the manometer fluid.

These requirements are met by using a monostable valve relay circuit which can be triggered into its unstable state when the manometer circuit is complete. A test of the

Fig. 3. Diagram of the manometer with its connexions to the respiration chamber and compensating volume.

Fig. 4. Diagram of the electrical circuit. The relays are Post Office type 3000, as follows: A/3 400 ohm coil; contacts (1) C.O., (2) make, (3) make. B/2 1000 ohm coil; contacts (1) break, (2) C.O. C/1 200 ohm coil; contacts (1) make. A/3 is adjusted so that A/2 breaks before A/1. Resistors are $\frac{1}{2}$ w carbon except those labelled 'w.w.' which are 10 w wire-wound.

manometer circuit is made at intervals of 1 min. by the 'timer', which consists of a micro-switch S1 operated by a cam driven at 1 rev./min. by the synchronous motor that also drives the chronograph.

In the stable condition the grid of valve V1a is more negative than the grid of V1b, and therefore current flows preferentially through V1b, the relay B/2 is energized and the contacts B1 are open. When the micro-switch S1 makes, the relay A/3 is energized, contacts A1 change over to connect the manometer while contacts A2 ensure that the circuit is in its stable condition. If at this time the electrode in the manometer is making contact with the meniscus, current flows through the manometer fluid and the capacitor C1 becomes charged. When the micro-switch S1 opens, the contacts A2 open first, then the contacts A1 connect the capacitor C1 to ground, thus placing a positive-going pulse on to the grid of V1a which causes this valve to pass current instead of V1b. The relay B/2 therefore de-energizes and the contacts B1 apply a voltage to the oxygen generators. The contacts B2 in conjunction with the relay C/1 energize pen solenoid P.S. 1 for a brief time to mark the chronograph chart.

After 1 min. has passed the micro-switch again makes and the relay contacts A2 close, restoring the circuit to its stable state and switching off the oxygen generators.

The current through the generators is adjusted by $R1 + R2$ to a value of 27.2 ma (equivalent to 0.1 ml. of O_2 /min. at 15° and 760 mm.).

A power supply unit (not shown) supplies the current for the relays as well as a 300 v regulated supply for the valves and the oxygen generators.

The chronograph was made for a 1.5-v supply [C. F. Palmer (London) Ltd.; six-pen model] and is used with the 6v supply by adding 5Ω resistors in series with the solenoids.

Procedure. The T taps on the manometer bridge (Fig. 3) are turned so as to interconnect the compensating volume, the manometer bridge and the respiration chamber. Sufficient sodium hydroxide solution (0.1–1.0N) to absorb all the carbon dioxide produced during the course of the run is put into the base of the respiration chamber, the plant material inserted and the lid sealed on and clamped down. The oxygen generator is connected to the respiration chamber and the whole unit (respiration chamber, manometer, compensator and generator) immersed in a constant-temperature water bath ($\pm 0.01^\circ$) and left to attain temperature equilibrium (1–2 hr.). At the end of this time the compensating volume and manometer are shut off from the respiration chamber and the latter is flushed with gas of the required partial pressure of oxygen, equilibrated to the temperature of the water bath and to a relative humidity of about that above the solution of sodium hydroxide in the respiration chamber. The respiration chamber is then finally closed to the atmosphere and by manipulation of the taps on the manometer unit the compensating volume is first brought to the same pressure as the respiration chamber and then connected to it via the manometer only.

The electrode in the manometer is set to just make contact with the meniscus when there is no pressure difference across the manometer, with the result that at least one pulse occurs when the oxygen generator and relay are switched on and thereafter the pulse rate settles down to correspond to the true respiration rate.

DISCUSSION

The respiration chambers so far used have had volumes of about 600 ml. and the addition of an oxygen pulse of 0.1 ml. has blown the meniscus in the manometer clear of the electrode. This pulse volume is relatively large for a slowly respiring tissue and could be reduced to some extent but the limiting conditions have not been studied.

Temperature fluctuations of the water bath must be very small. The temperature variations as the heater comes on and off are smoothed out by constructing the chamber of relatively thick insulating material. Without this the time of individual pulses would vary but an average of a number would give the true rate of respiration.

The manometer is only tested for electrical continuity at intervals of 1 min. and a pulse of oxygen is produced at the first whole minute after contact between the meniscus and the electrode has been made. This limits the accuracy over short periods. If this error were important the frequency of testing could be increased with a corresponding increase in current to the generator, but alteration in this direction is limited by the rate of absorption of hydrogen by the cathode.

For certain tissues, e.g. apples, the use of a closed chamber is unsatisfactory because of the accumulation of 'volatiles', but the effect can be virtually eliminated by periodically flushing the respiration chamber with the appropriate gas mixture.

At very low partial pressures of oxygen, particularly with actively respiring material, a magnetic stirrer should be incorporated in the respiration chamber to reduce the diffusive resistance of the system.

The accuracy of the apparatus has been tested by setting it up without plant material and connecting the tap on the roof of the chamber to a small gas pipette immersed in the water bath. The gas pipette was initially filled with mercury which was allowed to flow out slowly (1–2 ml./hr.). After 15–18 ml. had been removed the flow was stopped and the apparatus allowed to come to equilibrium. The volume removed was calculated from the weight of mercury removed and this was compared with the volume of oxygen produced by the external generator, allowance being made for water vapour pressure, temperature and barometric pressure changes. The recovery from the external generator in several experiments was within 0.1 ml. of the calculated volume of mercury.

SUMMARY

1. An apparatus for recording the oxygen uptake by whole plant organs is described. The respired carbon dioxide is absorbed by a solution of sodium

hydroxide and the oxygen taken up is replaced by electrolytically generated oxygen, which is added in small equal-sized volumes. The frequency of their addition is a measure of the respiration rate.

2. The oxygen is produced by electrolysis of a solution of sodium hydroxide by using a large palladium cathode to absorb the hydrogen.

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Studies in the Biochemistry of Micro-organisms

109. THE STRUCTURE OF ASPERTHECIN*

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Asperthecin, $C_{15}H_{10}O_8$, the polyhydroxyanthraquinone derived from *Aspergillus quadrilineatus* Thom & Raper, was first isolated by Howard & Raistrick (1955). They prepared various derivatives and advanced a partial structure based on emodin. As a result of further degradative work by Neelakantan, Pocker & Raistrick (1957), the structure of asperthecin was reduced to two possibilities, (I) and (II), but the evidence was insufficient to determine which was correct.

Further work on asperthecin, here presented, has afforded proof that the structure of asperthecin is represented by (II).

The evidence previously available, supporting structure (II), was mainly based on the visible absorption spectrum. The location of the main absorption band in this region at $\lambda_{484m\mu}$ (Birkinshaw, 1955) is strong evidence for the presence of three α -hydroxyl groups (cf. Birkinshaw & Gourlay, 1961). A further indication in favour of structure (II) was the fact that methylation of asperthecin with diazomethane in ether for a limited period afforded a di-*O*-methylasperthecin. Prolonged methylation in presence of methanol pro-

duced tetra- and penta-methylasperthecin, but a trimethylasperthecin was not isolated (Neelakantan *et al.* 1957). This suggests that asperthecin has only two β -hydroxyl groups.

The main evidence against structure (II) and favouring structure (I) was the fact that solutions of asperthecin showed no fluorescence in daylight. Since fluorescence was considered to be diagnostic for quinizarin derivatives the presence of a 1,4 arrangement of hydroxyl groups as in structure (II) was unlikely (Howard & Raistrick, 1955).

Further evidence favouring structure (II) has now been obtained by partial (short-time) acetylation with acetic anhydride, by the method used in investigating dermocybin (Birkinshaw & Gourlay, 1961). After 12 min. in boiling acetic anhydride asperthecin afforded mainly a red triacetate with some orange tetra-acetate. The first stage of acetylation should be confined to the nuclear β -hydroxyl groups and the ω -hydroxyl group. Since a triacetate is first formed, the inference is that asperthecin has only two β -hydroxyl groups and hence three α -hydroxyl groups. In accordance with this, triacetylation of asperthecin produces only a small shift in the main visible absorption maximum from 484 to 478 $m\mu$, the latter value being still

* Part 108: Stickings & Townsend (1961).

(I)

(II)