

# Automatically Recording Respirometer

ROBERT M. ARTHUR

*Bioengineering Laboratory, Rose Polytechnic Institute, Terre Haute, Indiana*

Received for publication 5 June 1964

## ABSTRACT

ARTHUR, ROBERT M. (Rose Polytechnic Institute, Terre Haute, Ind.). Automatically recording respirometer. *Appl. Microbiol.* **13**:125-127. 1965.—Recently, several different types of devices have been developed which automatically record oxygen consumption of biological cultures. This article describes one of these devices and discusses its application.

Manometric methods have long been used to measure oxygen consumption by biological cultures. In fact, most of the existing information on cell respiration and metabolism has been obtained by the use of manometric methods and devices. In spite of this, and the fact that several authors (Umbreit, Burris, and Stauffer, 1959; Jenkins, 1960) have in the past pointed out that automation would be a desirable feature of manometry, the development of automatic methods has been slow. Today, three types of automatically recording respirometers are being marketed, and several others are in the development stage.

Two automatic devices were described and discussed in the latest edition of *Manometric Techniques* (Umbreit et al., 1959). Both of these devices are available commercially. A third device, also available commercially, was demonstrated at the 48th Annual Meeting of the Federation of American Societies for Experimental Biology. Two other devices have been described in the literature (Arnold, Burdette, and Davidson, 1951; Clark, 1959).

The major difference in the devices is in the method of sensing the change in partial pressure of oxygen. All of the devices use some type of transducer to convert the change in pressure to an electrical signal which can be fed into a potentiometric recorder. Most of the devices can be directly connected to conventional respirometers.

The device described herein accomplishes the same results as the above-mentioned devices, but is less complicated in operation. A schematic diagram of the device is shown in Fig. 1. It operates in the following manner. As the culture in chamber (A) utilizes oxygen and the generated CO<sub>2</sub> is absorbed by alkali, the partial pressure of the oxygen in the atmosphere decreases, causing the manometer fluid in the closed leg (B) of the

oil manometer to rise. This in turn causes the fluid in the open leg (C) of the manometer to fall, thereby changing the position of the float (D) and the armature of the linear differential transformer (E). As the armature is displaced, dissimilar voltages are impressed across the two secondary windings in accordance with iron core transformer theory. The difference in the magnitude of the secondary voltages is directly related to the displacement of the armature, providing that the displacement is not larger than a prescribed amount for the particular transformer model. Outside this range, the relationship between the impressed voltage and the displacement of the armature is not linear. The a-c signal from the transformer must be rectified to d-c before being fed into the recorder (F). The d-c signal which is fed into the recorder is itself proportional to the displacement of the armature. The result is a record of change in oxygen partial pressure with time, i.e., an oxygen-consumption curve.

The present device is calibrated so that full scale on the recorder equals 100 ml. When the total consumption of oxygen equals 100 ml, valve G is closed and valve H is opened manually. Oxygen is forced into the manometer by compressing the collapsible bag (I) containing pure oxygen. This causes the level in the closed leg of the manometer to fall and the level in the open leg to rise, moving the recorder pen back to zero. This step could be avoided by using a larger manometer flask. The procedure could be facilitated by the use of automatic valves controlled by the recorder pen.

The device is easily calibrated by adjusting the voltage applied to the primary winding of the transformer. In practice, the recorder is adjusted to zero by adjusting the null control on the rectifier circuit, adjusting the liquid level of the manometer tank, or adjusting the vertical posi-

tion of the transformer. A known volume of manometer fluid is then withdrawn from the open manometer tank. The recorder will then indicate a reading which may or may not be similar to the amount of fluid withdrawn. If the recorded amount is equal to the amount withdrawn, then calibration is complete. If not, an adjustment of the input voltage will either increase or decrease the recorded value. It is then necessary to repeat the zeroing and the calibration, because voltage adjustments affect the position of the null. In any event, it is usually desirable to check both the full and half range of the instrument.

The range of the instrument is easily changed by any factor, either by changing the input voltage by the inverse of that factor, or by changing the cross-sectional area of the open leg of the manometer by the same factor. The first method is faster but may be limited by the range of linearity of the particular transformer used. The second method is preferred because the range of

armature movement of the transformer is not altered.

More than 100 tests were made to determine the accuracy and precision of the device. These tests were made by comparing the theoretical total oxygen demand of a sodium sulfite and cobalt catalyst solution to the experimental oxygen demand as indicated by the device. Because sodium sulfite exerts an immediate and high demand for oxygen, some difficulty was experienced in the introduction of sulfite to the closed chamber. This resulted in an experimental oxygen demand which was smaller than the theoretical demand. Eight different procedures were tried in an effort to eliminate this source of error. If the results of all of these procedures (104 runs) are analyzed, the average experimental demand was 83.0 ml (STP) of oxygen. The theoretical demand was 89.0 ml of oxygen. The experimental results were about 7% in error. The standard deviation for the experimental results was 7.29 ml. One of the eight procedures resulted in an error of 0.68% and a standard deviation of 4.24 ml. The latter results indicate that the device is both precise and accurate. Complete results and the method of running these tests were given by Arthur (1963).

For all practical purposes, the device operates at constant pressure. In other words, the difference in elevation of the levels of oil in the closed and open leg of the oil manometer is small. In its present application, the difference in elevation is 1.29 cm of water when 100 ml of oxygen have been utilized. It would be almost impossible to observe this small change, accurately, by the naked eye. The use of the linear differential transformer makes this change and even smaller changes easy to observe by converting it to an

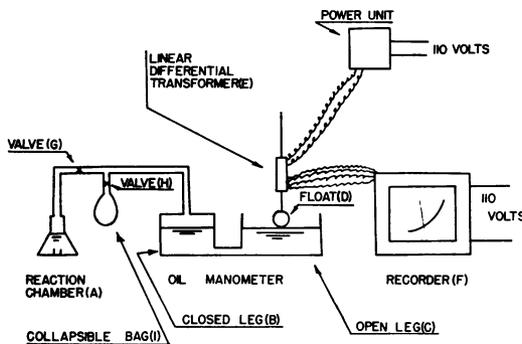


FIG. 1. Schematic diagram of automatically recording respirometer.

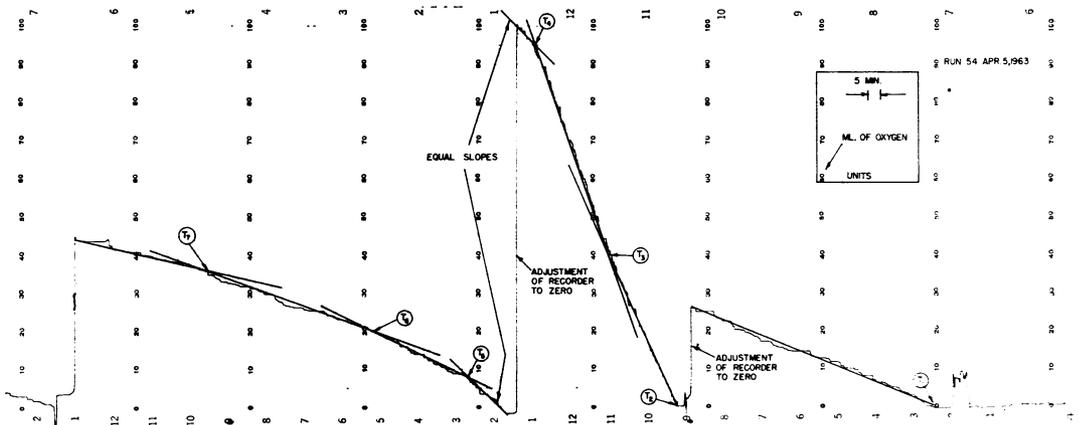


FIG. 2. Graph of oxygen uptake by a culture of *Pseudomonas fluorescens*.

electrical signal which can be amplified and recorded. By suitable selection of the cross-sectional area of the open leg of the oil manometer, this change in elevation (pressure change) can be kept small and within the linear range of a specific transformer for any volume of oxygen demand. Staying within the linear range presents no problem, for transformers with a variety of ranges are available.

The device can be easily connected to any existing respirometer by merely connecting the reaction vessel to the closed leg (B) of the oil manometer. For large-scale work, the reaction vessel can be increased to any desired size. To assure maximal gas exchange with large volumes, it may be necessary to either recycle the gas from the top to the bottom or recycle sample from the bottom to the top of the reaction chamber.

An example of the use of the device to measure the oxygen uptake of a culture of *Pseudomonas fluorescens* is shown in Fig. 2. The culture was inserted in the reaction chamber at time  $T_1$ . At time  $T_2$  a weighed amount of glucose was added. The resulting increase in oxygen uptake is clearly evident. In addition, every other variation in the uptake is dramatically displayed, as evidenced by the breaks in the curve at times  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$ , and  $T_7$ . Uptake rates and total consumption are easily computed from the graph. Corrections for variations in pressure or temperature can be made in the usual manner or can be compensated

for in the original calibration of the device. Sample sizes from 10 to 1,000 ml have been used with the present device.

The advantages of the device over present manual methods of respirometry include the following: (i) automatic and continuous recording of gas exchange in a biological process; (ii) the use of samples of various sizes; (iii) accuracy in determining the magnitude or rate of exchange; (iv) provides a complete record of all variations in exchange rate without tedious data taking and plotting; (v) easily adjusted range; (vi) adaptability to present methods.

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