

which pass three glass tubes: a short one just enters the jar, another one reaches about half way down, and the third goes to the bottom of the chamber. Air is circulated through the system by a simple diaphragm pump with glass valves which is operated by a vari-

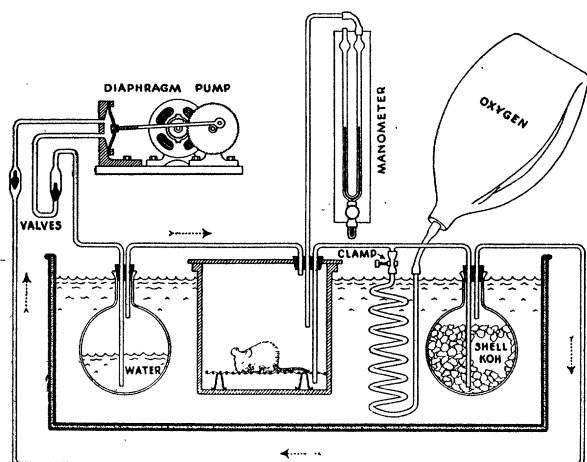


FIG. 1. Diagrammatic sketch of manometric respiration apparatus.

able speed electric motor adjusted to deliver approximately a liter of gas per minute. The air bubbles through a flask of water, enters the top of the animal chamber through the short tube, and is taken out at the bottom by the longest tube. This removal of the bottom layer of gas prevents CO_2 from accumulating in the animal compartment. Carbon dioxide is removed from the air as it passes through the flask containing shell potassium hydroxide, and the dried, CO_2 -free gas is then returned to the pump and re-circulated. The third tube from the animal compartment leads to a Warburg manometer containing Brodie solution.¹ Changes in the height of this fluid column indicate pressure variations in the system. A T connection to the gas line leads through a coil to a rubber anesthesia bag which contains oxygen for refilling the system. The animal compartment and the flasks are nearly submerged in a constant temperature water bath.

Since the manometer has one end open to the air it is necessary to correct the readings of the fluid column for changes in barometric pressure. This is done with a second manometer, not shown in the sketch, which is connected to a second chamber of approximately the same volume as the total gas space in the first system. This vessel is immersed in the water bath and therefore will compensate for slight temperature changes also.

Before making an oxygen consumption determination, the apparatus is checked for leaks by observing

¹ M. Dixon, "Manometric Methods." Cambridge. 1934.

whether it will maintain a positive or negative pressure. The animal is then placed in the chamber in the water bath at 28°C , connections with the air flow line and manometer are made with rubber tubing, and the pump is started. During an initial ten-minute equilibration period the manometer stopcock is closed and the clamp on the line leading to the oxygen bag is left open; since there is a slight positive pressure due to the elasticity of the bag, there is a flow of gas to compensate for any oxygen consumption by the animal. This clamp is closed before manometer readings are started. When the manometer fluid falls to the bottom of the scale more oxygen is admitted by opening the clamp and the fluid is relevelled to the 150 mm mark. The system may then be closed and read again within one minute. Since the oxygen is thus quantitatively replaced there is never a significant deficit.

Readings and thermobarometric corrections are made as in the Warburg type of manometer,¹ and a constant for the apparatus may be calculated in the same way if the volume of the system is measured. A simple multiplication of the corrected reading by the constant then gives the volume of oxygen adjusted to normal temperature and pressure.

Since the sensitivity of the system depends upon the gas volume, it is possible to adjust it by altering the size of the animal chamber. The device as illustrated with a volume of about seven liters is sufficiently sensitive to permit readings each minute with an adult rat.

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A MAGNETIC STIRRER FOR CONTINUOUS GAS-FLOW APPARATUS

IN the course of growth studies on the alga *Chlorella*, it was found desirable to compare growth in continuously stirred cultures with that in intermittently stirred cultures. In the latter, 5 per cent. CO_2 in air was bubbled through eight flasks in series. To maintain analogous gas conditions, a simple magnetic stirrer was devised which permitted the same stream of gas to flow through the series of cultures. The accompanying diagram (Fig. 1) shows the essential features of the apparatus.

A constant-temperature bath having a shoulder with a glass inset bottom (A) is employed. In the space below the shoulder is a bank of Mazda lamps (B) that may be adjusted in elevation to vary the intensity of illumination. The lamps are cooled by a fan; and the bath temperature is regulated by a thermostatically controlled heater and refrigerator coil (not shown).

A horizontal rod (C) is fastened to the front of the bath, and from this eight Erlenmeyer culture flasks are supported in the bath over the glass shelf by means of jaw clamps. Each flask is one-third filled with liquid medium containing the algal cells. The gas mixture of 5 per cent. CO₂ in air from a cylinder of compressed gas is bubbled through the vessels connected in series by glass bends and rubber tubing.

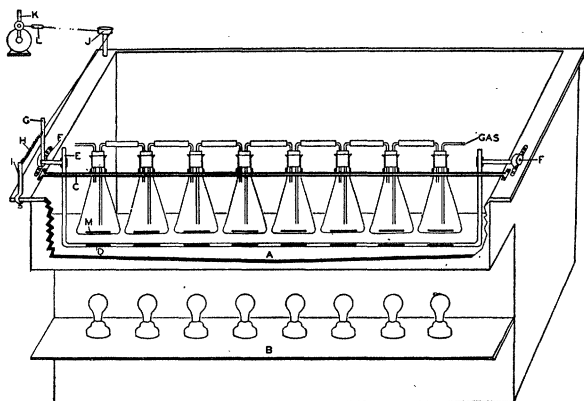


FIG. 1

Stirring of the cultures is effected by paired cylindrical Alnico magnets (4 mm × 45 mm). Eight driving magnets (D) are inserted into a glass tube (4.5 mm bore) with like poles facing, and the tube (E) is bent into a U to fit into the bath. The magnets will tend to distribute themselves equidistantly, but can be moved to desired positions by an externally applied magnet. The U-tube is supported in the bath by clamps attached to wobble bearings (F). Each bearing is made by bending a strap of brass to fit around a roller-skate wheel and then bolting the two together. The lateral extensions of the brass strap are then screwed or clamped to the sides of the bath. These bearings require no lubrication, are practically wear-proof and allow considerable tolerance in alignment of parts. Fastened between the left bearing and its clamp is an upright brass plate (G) (6" × 1") with a series of small holes drilled along both edges. A light spring (H) is hooked through a hole in the front edge and connected to a vertical clamp (I) at the corner of the bath. A loop of wire is passed through a hole at the other edge and joined by a braided cord and pulley (J) to an eccentric drive turned by an electric motor. The eccentric is made by attaching a brass bar to the shaft of a reducing gear connected with the motor, drilling a series of holes in the bar, and fastening a roller-skate wheel to it by a wing nut and bolt through one of the holes. The pulley cord is connected to the wheel by a wire loop, and a turn-buckle (L) is inserted to take up slack.

The stirring magnets are similar Alnico cylinders.

Each is sealed in Pyrex tubing to render it non-reactive with the cultures. The tubing is thick-walled to withstand mechanical shock, and is thoroughly annealed to eliminate strain. One such stirrer (M) is inserted into each flask of medium prior to sterilization and inoculation. These stirring magnets automatically assume proper polar orientation with respect to the driving magnets.

In operation, the turning of the motor causes the U-tube with the driving magnets to swing as a pendulum under the flasks. The driving magnets in turn impel the stirring magnets to roll back and forth in the culture flasks, setting up mixing currents. An oscillation rate of about one swing per second has been found to keep even the heaviest cultures in indefinite suspension. The rate is most readily adjusted by a rheostat in series with the motor. The amplitude of the swing can be regulated by changing the position of the wheel on the motor eccentric and by adjusting the ratio of the length of the brass plate (G) (from attachment to bearing) in relation to the length of the vertical part of the U-tube (from bearing to bend).

This magnetic stirring arrangement has several advantages over the usual propeller or pump stirrers or shakers. Mechanically, it is simple to construct and operate. Continuous serial gas flow may be maintained through the vessels. Gas connections do not have to be wired to prevent their shaking loose. Single flasks may be removed for sampling without interrupting the agitation of the others. Since stirring is effected within a sealed system, the purity of cultures can be maintained. Actual use has shown that the stirring is gentle but effective and causes no evident damage to the cells. On the contrary, the improvement in the cultural conditions is such that more than double the yield is obtained in half the time. Though originally designed for algal-growth experiments, this magnetic stirrer may well find use in a wide range of biological and chemical procedures in which continuous gentle agitation is desired.

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