Technical methods

A pH stat for carbon dioxide incubator control

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Commonly used methods of controlling the pH of bicarbonate-buffered tissue cultures within an incubator are inaccurate and extravagant in carbon dioxide. Moreover, technical problems which may result in the loss of cultures are common. The instrument described has been designed to measure the pH of a solution containing the same concentration of bicarbonate as the medium in which the cells are being grown, and to use these data to control the amount of carbon dioxide admitted to the incubator. Hence the pH of the culture will follow that of the measuring system.

Observations are presented which demonstrate superior accuracy of control of pH within the culture medium, and much lower carbon dioxide consumption than that of other methods.

Common methods of controlling carbon dioxide incubators

Two main methods of controlling the carbon dioxide concentration inside incubators have been used. They are: (1) the purge method, where a calculated volume of carbon dioxide is added to provide the correct final concentration; and (2) the continuous-flow method, where carbon dioxide and air are introduced into the incubator continuously in the required ratio. Each of these methods has disadvantages, and technical failures can result in the loss of valuable cell cultures. Problems associated with the purge method are that the duration of the door opening is variable, hence the atmosphere left within the incubator after the door has been closed is of extremely variable composition. When the incubator is left unattended for a long period of time, for example overnight, leakage of gas from the incubator allows the carbon dioxide concentration to fall, although Wright (1964), who designed the purge method, made provision for leakage from the incubator by incorporating a small continuous flow of carbon dioxide into the incubator to com-

pensate for losses. The continuous-flow method is unaffected by leakage; indeed, it requires leakage, but precise regulation of the gas flow is essential, and the needle flow control valves tend to clog and alter the ratio of the gas mixture. This method also involves an extremely high consumption of carbon dioxide.

More recently, incubators have been introduced that use a feedback control system operating from a thermal conductivity cell in the cabinet. These were not commercially available at the time of this study, and although they are undoubtedly efficient, it is felt that a system in which pH is directly monitored and controlled offers both theoretical and practical advantages over a thermal conductivity control system. From a theoretical viewpoint since pH is the crucial variable it is preferable to control it directly. As a practical matter, the circuit design of the pH stat was simpler than that required for the conductivity cell. It is necessary only to use the same buffer in the measuring chamber as in the cultures to obtain the correct pH response, and routine calibration is much simpler with the direct pH system.

In the present study, measurements of pH were made in 4 ml of 25 mM bicarbonate buffer solution placed in a 30 × 10 mm tissue culture dish using a Radiometer microelectrode and an EIL Laboratory pH meter. The pH electrode and dish were mounted in a small jig to maintain their relative positions and placed in the centre of the incubator. Figure 1 shows the results obtained with a Leec Incubator Type GF2 using the two common methods described. Clearly, the purge method showed inadequate control of pH even over a short period of time, and although the continuous-flow method was effective, its high cost and inherent unreliability made a new trouble-free and inexpensive system very desirable.

Design of the pH stat

Since the actual requirement is to maintain the correct pH in the tissue culture fluid itself, a system which measured pH directly was chosen. Unless elaborate expensive seals are used, incubators will always leak to the atmosphere. This led us to use pure carbon dioxide to maintain the correct level (Carpenter and Prater, 1964). Figure 2 shows the layout of the system designed. A small aquarium pump (HYFLOW 0.9 l/min) aspirates the atmos-

Fig. 1 Comparison of continuous-flow and purge methods of gassing a carbon dioxide incubator during a 24-hour period. Measurements were made in 4 ml of 25 mM sodium bicarbonate solution in a 30 × 10 mm culture dish, as described in the text.

phere from the incubator and bubbles it through a condenser and then a measuring chamber. The condenser is required to remove excess water vapour when the incubator is operated at high humidity and incorporates a capillary overflow pipe to return water to the incubator. Without the condenser, the buffer solution in the measuring chamber gradually becomes diluted, which would alter its pH relative to that of the cultures. The measuring chamber contains a small volume (sufficient to cover the pH electrode) of 25 mM sodium

bicarbonate buffer solution containing a few drops of phenol red to provide a visual indication of pH. A combination pH electrode (EIL Laboratory Model 1600) is immersed in the buffer solution, and continuous measurement is made and displayed by the pH stat. Figure 3 shows the schematic circuit diagram of the pH stat. The use of integrated circuit amplifiers has made the construction both simple and reliable. A field effect transistor input operational amplifier has a particularly high input impedance (10^{14} ohms; RS components FET MOPA) which makes it highly suitable for use with pH electrodes. Amplifier A1 operates as a voltage follower (unity gain) and its output is fed to a low pass filter. The filter prevents false triggering of the level detector caused by noise pickup. A2 is a variable gain amplifier and A3 a level detector. The output from the level detector operates the solid state relay, which in turn energises the solenoid valve. Amplifier A4 (unity gain) is provided to drive the output meter, and a biased switch on its input allows display of the set pH value.

Operation

If the pH of the buffer solution in the measuring chamber is higher than the set value, the solenoid valve opens, allowing the entry of carbon dioxide into the incubator. A very fast response is ensured by connecting the gas inlet to a T-piece, which is

Fig. 2 Layout of component parts of the pH stat (not drawn to scale).

Fig. 3 Schematic circuit diagram of the pH stat.

common to the sampling port. Hence, when carbon dioxide is entering the incubator, some of it is drawn into the sampling line and passed on to the measuring chamber, where its effect upon the buffer solution is very rapid and causes the solenoid valve to shut. The carbon dioxide entering the incubator quickly equilibrates with the existing atmosphere, the pH of the buffer rises, and the gas valve opens again. After a few similar cycles the control value is reached. Using this technique, pH overshoot is greatly minimised, and the carbon dioxide concentration returns to the correct value within 8 minutes. It is important to note that the response time of the measuring system has been made many times faster than that of the cultures ($20\times$).

In practice, carbon dioxide is admitted once every two hours when the incubator is left closed.

Calibration

For convenience of meter scaling and useful range, a span of 2.5 pH units was chosen from pH 5.75 to pH 8.25 with pH 7.00 as the central value. The EMF generated by the pH electrode chosen was zero at pH 7.00 with a slope of 59 mV/pH unit at 25°C, thus giving a full span of 147.5 mV. The zero control of A1 (available on the front panel as the set buffer control) was offset to provide zero at the output of A1, for an input of -73.75 mV, so that all voltages after A1 were unipolar. The set

slope control was adjusted to give full scale (8.25 pH) on the output meter when the input to A1 was $+73.75$ mV. The set pH potentiometer was arranged to provide the same voltage swing as that appearing at the output of A2. The pH electrode was then connected to A1 input and immersed in a standard buffer solution at pH 7.00. The set buffer control was adjusted to read pH 7.0 on the meter. Fine adjustment of the set pH value control alters the voltage applied to the level detector and hence the point at which the valve operates.

Accuracy

The desired accuracy of pH control for tissue cultures is ± 0.1 pH unit, which in terms of electrode voltage is ± 5.9 mV. The combined noise and drift figure for A1 was better than $10\ \mu\text{V}$, and A2 operated at a gain of 10 gives an output noise and drift of approximately $100\ \mu\text{V}$. These values are insignificant. The reproducibility of the pH electrode immersed in a pH 7.0 buffer was ± 2 mV or ± 0.03 pH unit. Operating the electrode without a temperature compensator introduces an error of ± 0.11 mV/°C, which is equivalent to 0.03 pH unit between 20°C and 37°C. The largest error in the system was therefore the electrode itself, but even so the combined errors of the system were ± 0.06 pH unit, which is well within the ± 0.1 pH unit required.

Performance

The performance of the completed system was evaluated in the same way as for the other methods of carbon dioxide control, and the results showed a marked improvement (Fig. 4) of control after a 24-hour period. The average difference between the measured pH recorded by the meter and that recorded from the pH stat was 0.032 pH unit. The consumption of carbon dioxide of all three systems was measured using a calibrated rotameter

Fig. 4 Results obtained when using the pH stat to control the gassing of a carbon dioxide incubator. Measurements made by the same technique as for Figure 1.

flow meter. The carbon dioxide consumption was considerably less using the pH stat, being 17 litres/day compared with 144 litres/day using the continuous-flow method and 90 litres/day using the number of purge operations necessary to maintain the pH within the ± 0.1 pH unit required.

Conclusions

The system described provides a high degree of control over the pH of tissue cultures within an incubator. Various cell types have been cultured over a three-year period, including mouse bone marrow cells, Chinese hamster lung fibroblasts, and human lymphocytes. No technical faults have occurred. The system is inexpensive to produce and can be fitted to any carbon dioxide incubator. The system fails in the safer condition, that is, when the carbon dioxide supply is off.

References

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