range of only 2 c.p.m. It is always possible to check the background of a particular sample by allowing time for a calculated decay of the rubidium-86 contribution to the total activity, and then recounting.

Time of sampling

After injection of the radioisotope into the above-ground parts of the plant, sufficient time must elapse to allow the isotope to be distributed uniformly within the roots. Scott Russell and Ellis (1968) used barley plants grown in nutrient solution to investigate the speed of translocation of the isotope and its evenness of distribution. They found that little rubidium was lost from roots and that some accumulation in root apices occurred, but this was not a serious source of error. Growing large vegetable plants to maturity in solution culture is difficult and therefore, to investigate the speed of translocation, we grew plants in large plastic pots in which a small "window" of thin polythene was made. The proliferation of roots against the surface of the container gave a high root density without intervening soil. The gas-flow Geiger counter taken from a Tracerlab Chromatogram Scanner was placed in contact with the polythene window. The connections were maintained to the recording ratemeter of the scanner, enabling the movement of radioisotopes into the roots of the plant to be monitored. The isotope was injected into the aerial parts of the plant which were shielded from the counter cell. With lettuce, Brussels sprout and cabbage plants the isotope was detected 2 to 3 hours after injection and came to equilibrium in from 30 to 40 hours. A minimum time of 3 days was allowed therefore between injection and sampling.

In an experiment to investigate the rooting pattern of lettuce, the petioles of a mature plant were injected with 24 microcuries of rubidium-86 in a volume of 80 microlitres of water.

Soil samples 2.5 cm diameter and 7.5 cm deep were taken with a sampling tube 3 days later in the positions shown in Figure 1. The samples were analyzed as described above and the results are shown in Figure 1. The contour lines, representing 10, 32, 100, 320 and 1000 c.p.m. were obtained from a plot drawn by a computer programme written by Ross & Lauckner (1971) of Rothamsted Experimental Station Statistics Department. The very high figure for the sample nearest the plant is due to the inclusion of the 'tap root' which is not representative of true root tissue and usually discounted.

The contour diagrams of root distribution obtained by this method are especially suited to the investigation of the root system of individual plants, for example of a single crop plant separated from its nearest neighbours in the row and in adjacent rows by wide row spacing and wide inter-row distances. The technique could equally easily be applied to the investigation of the distribution of roots around a row of closely spaced cereal plants where the variation in two dimensions only need be considered, or to the distribution of roots below, for example, a grass sward, where only the change of root density with depth need be measured. The method has the main advantage that commercially available equipment may be used for the measurement of radioactivity in the soil samples.

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Apparatus and devices

Low cost multichannel scanning pH-stat

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Introduction

The cultivation of micro-organisms under controlled conditions is a standard procedure in many microbiological laboratories. In order to obtain such conditions, especially when organic acids or reduced sulphur compounds are used as energy sources for growth (Harder and Veldkamp 1967; Kuenen and Veldkamp 1970), pH control of the cultures is obligatory. Until recently this has been accomplished in our Department by using separate pH controllers for each culture. However, as a result of the cost of individual pH controllers, an ever increasing demand for pH control in bacterial cultures could not be met. It was therefore decided to develop a scanning pH-stat which, with the aid of only one pH controller, would control the pH in at least 12 cultures. This communication reports the construction and use of such a scanner which already has been used successfully for several years.

Description

In principle the instrument consists of a commercial on/off pH controller (Analytical Instruments, Model RZ1 SN 4-10) connected to a generator giving a pulsating DC-voltage which operates a magnetic valve. Opening the valve results in the addition of either alkali or acid to the culture. The scanner functions as a multiposition switch connecting a specific pH electrode to the corresponding magnetic valve (Figure 1). Depending on the capacity of the switch any number of electrodes and magnetic valves can be accommodated. In our design this number has been limited to 12, allowing pH control in 12 separate bacterial cultures. The working principle of the scanner is as follows (Figure 2). A time basis generator (T_4) operates a solenoid (L_1) which is connected to a 12 position switch (S_1) equipped with 4 decks. The cycling time of the generator can be adjusted and, as a result of the generated voltage, switch S_1 is moved one step forward. Deck S_{1a} of the switch selects one of the pH-electrodes. The corresponding magnetic valve is chosen by deck S_{1b}, which connects a multivibrator (T_3) *via* a one-shot (T_2) to the power amplifier which operates the valve. The multivibrator used has an adjustable frequency of 0.5 to 3 Hz, while the one-shot can be adjusted from 0.05 to 0.4 sec. The power amplifier thus produces a voltage signal which is time and frequency controlled, allowing accurate adjustment of the volume of reagent delivered by the valve. Deck S_{1c} is used to skip switch positions which are not in use, while deck S_{1a} operates signal lamps indicating the position of the switch.

When switch S_2 is in the position "manual", the time basis generator T_4 is switched off. By using push button S_3, the scanner can then be set to any required position by skipping the other positions step by step. It will stay in the chosen position indefinitely, thus enabling continuous control of pH in any of the 12 positions.

When S_2 is in the position "automatic", switch S_1 is moved one position forward after a given time interval. This interval depends on the setting of the time basis generator and can be varied from 10 to 30 seconds. The instrument then scans each of the 12 positions during the interval chosen with a cycling time of 2 to 6 min. respectively. By using skip-switches it is possible to pass positions not in use, thus reducing the cycling time. It has been found that it takes a short time for the pH controller to adjust to the new pH value after S_1 has moved to the next electrode. During this time the reading is unstable and the magnetic valve should

not be operated. This safeguard has been achieved by using a dead-time generator (T_1) which blocks AND-gate P during an adjustable time-interval of 1.5 to 4 sec. This generator, which overrides P, is switched on by a Reed relay which is powered together with L_1. Thus the titrator is not operating during a short period immediately after switching from one position to another. The time needed for the slowest electrode to settle down is recorded when the generator is at 4 sec and the correct dead-time is selected accordingly.

The pH controller used is equipped with a single set point which can be adjusted manually. When the measured pH value deviates from the set point pH, switch S_4 is operated. At a given set point position, S_4 will be connected to C_1 when the actual pH is lower, while it will be connected to C_2 when the pH is higher than the set point value. Using switch S_5, C_1 or C_2 is connected to gate P, thus enabling either up-scale or down-scale adjustment of pH. As the controller has a single set point, pH control will lead to a common final pH in each of the 12 positions used, which would present a serious limitation to a wider application of the scanner. However it was possible to overcome this disadvantage by using an adjustable offset voltage of − 40 to + 40 mV in series with the reference electrode of the combined glass/reference pH electrode used. In this way the indicated pH on the controller can be made to differ ± 1 pH unit from the actual pH value measured. This allows pH control in any of the 12 positions at a value in the range of 6–8 pH when the controller is at pH7. This covers the range most frequently used in growing micro-organisms in our laboratory. It should be noted that in the present design control action is in one direction only. As a result the scanner will only control pH in processes in which the pH tends to deviate in a similar fashion (either up-scale or down-scale). If control action in both directions is necessary, the present design can be easily adapted for this purpose by using a 5-deck switch instead of the 4-deck switch S_1. The fifth deck is then used to select for each electrode either the up-scale or the down scale position on switch S_5.

To illustrate the working principle of the scanner outlined above, a schematic time-function diagram has been drawn (Figure 3). It has been assumed that the indicated pH deviated from the set point pH at the start of the control period (t_0), so that the magnetic valve controlling addition of either alkali or acid was activated. At time t the set point pH was reached. At t_0 the time basis generator (T_4), set at a cycling time of t_4 sec, is activated together with the dead-time generator. After a preset time t_1 of the dead-time generator, a voltage change at c of AND-gate P opens this gate and the resulting signal at q is led to the magnetic valves *via* the one-shot and the power amplifier. The frequency and duration of this signal, which are determined by t_3 and t_2, are adjustable on the multivibrator and the one-shot respectively.

In constructing the scanner it was found to be necessary to shield the entries of all the electrodes from electrical fields, including that of the pH controller. In addition the entries of the magnetic valves were shielded and the shields connected to earth, as well as all other metal parts including the body of switch S_1.

Operation

At the start of an experiment the magnetic valves and electrodes were connected to the scanner. The valves used were similar to those described by Lelieveld (1971). Combined sterilizable electrodes were purchased from Ingold, Switzerland or Electrofact, The Netherlands. The channels not in use were disconnected

Figure 1.

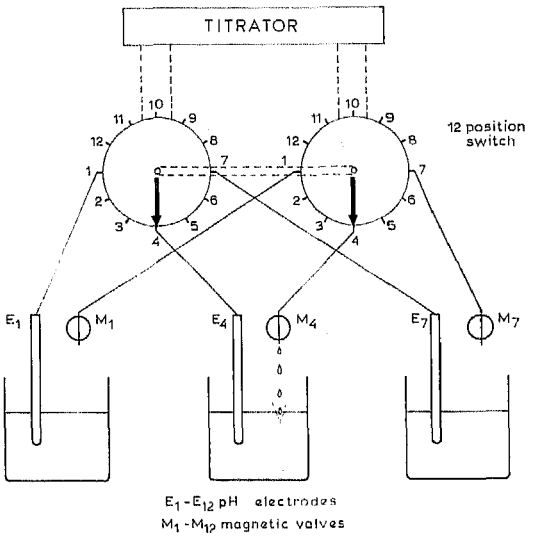
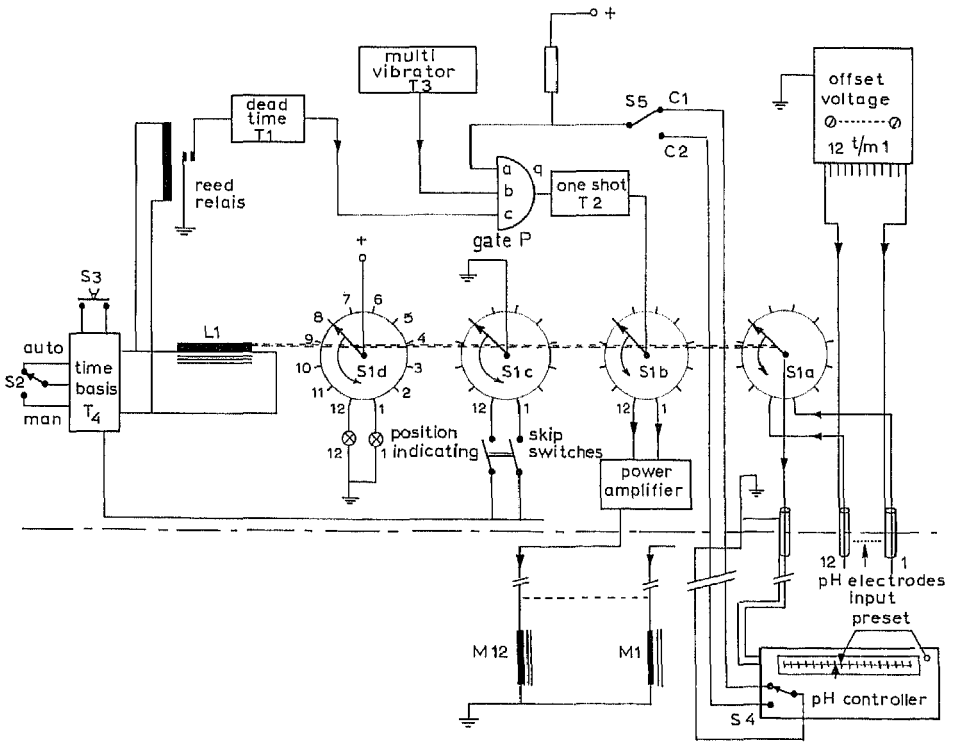


Figure 2.



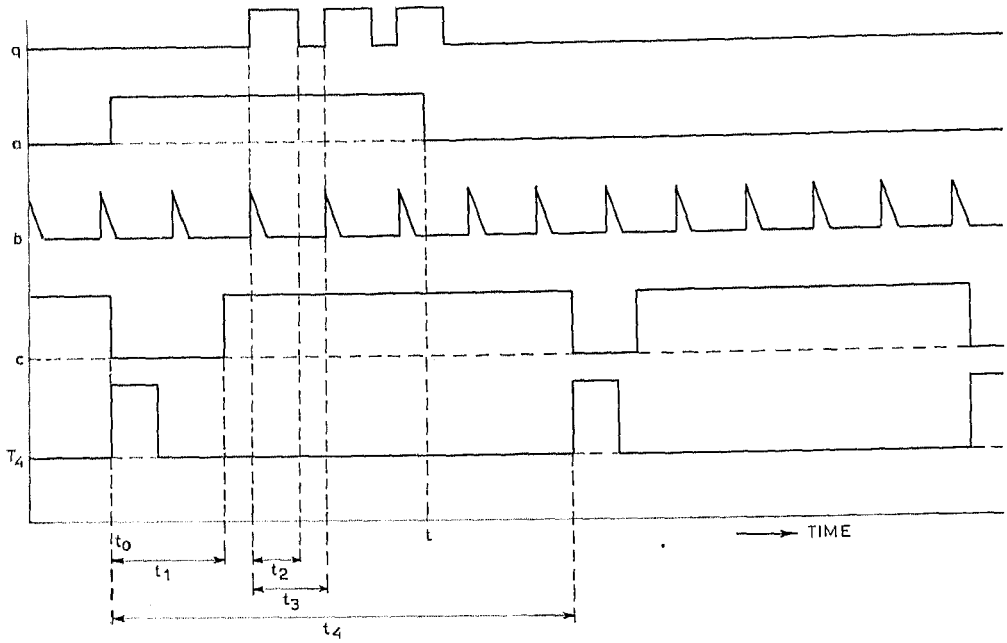


Figure 3.

by setting the corresponding skip-switches. With switch S_1 in the position "manual" and the set point indicator on the controller adjusted to the average value of the final pH values required, the final pH at each position was made to equal the set point pH by varying the offset voltage. This was done in the following way. All electrodes were placed in buffers having a pH similar to that required for that particular position. Push-button S_3 was then operated to select this position and by adjusting the offset voltage the pH value indicated on the controller was made to equal the set point pH. This procedure was repeated until all positions used were calibrated. Then the measuring time was chosen by adjusting the time basis generator. The optimal time was found to depend on the number of positions in use and the rate of change of the pH value in each individual position. Finally the dead-time was estimated as described earlier. In our experience a dead-time setting of either 2 or 4 sec generally was convenient.

The scanner is then ready for use. It is switched to "automatic" and started by operating push-button S_3. The scanner has been found easy to handle, even by inexperienced operators, and has been essentially trouble-free during more than 2 years of operation. A detailed circuit of the scanner is available from the authors on request.

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Apparatus and devices

Receiving acid for large-scale macro-Kjeldahl distillations

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Boric acid solution, in strengths ranging from 1% to full saturation has been used for the absorption of ammonia during Kjeldahl distillation ever since its introduction by Winkler (1913). Most of the factors affecting its use are discussed by Bradstreet (1965) in his excellent monograph on the subject of the Kjeldahl test. The protein-testing laboratory of the Canadian Grain Commission at Winnipeg annually carries out about 250,000 tests on wheat and other grains. There are 48 digestion and distillation units. The macro-Kjeldahl system is used, with a 1 g sample, 20 ml of sulphuric acid for digestion, and distillation into boric acid solution. The operation is semiautomatic, and analyses are performed by an assembly-line type of process. In this manner, a staff of four can comfortably effect 480 analyses during a single 7½ hour working day. Extensive use is made of custom-prepared equipment and chemicals.

"Receiving acid solution" is the solution into which liberated ammonia is distilled prior to the final titration, and consists of boric acid, water and a mixed indicator. Boric acid is best used with a mixed indicator. Many laboratories prefer an indicator consisting of methyl red and methylene blue. In our experience certain difficulties have been encountered in connection with the stability of solutions containing methylene blue, and an indicator solution consisting of methyl red and bromocresol green has been developed for use in our own operation. The colour of solutions of boric acid containing ammonia is green in the presence of the indicator, and the colour sequence at the end-point is green – greyish purple – purple – purplish red – reddish purple-red, and takes place over a pH range of 6.4 to 4.4. The true end-point occurs at a pH of 4.9 when the indicator is used for the titration, with standard sulphuric acid, of solutions containing ammonia.

The most suitable concentrations of indicator have been found to be respectively 3.5 ml of a 1% solution of bromocresol green,

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