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A microcomputer-based method for semi-continuous monitoring of biological activities

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Summary

A method by which a microcomputer can be used to monitor bacterial physiology by means of various electrodes (including oxygen, redox and ion-specific electrodes) is described and an outline of the software being used is given. A sample experiment, taken from the authors' studies on aerobic denitrification, is shown.

Key words: *Aerobic denitrification – BBC Computer – Ion-specific electrodes*

Introduction

During studies on *Thiosphaera pantotropha*, a facultatively chemolithotrophic sulphide oxidizing bacterium [1], it was found that this species was an aerobic denitrifier [2, 3]. In the early stages of the work, single parameters (e.g., oxygen consumption, nitrate reduction) were measured in separate experiments. However, in view of the controversy surrounding the actual occurrence of aerobic denitrification, it became important that simultaneous oxygen and nitrate reduction be shown in the same sample. It was therefore decided to develop a simple system for combining and monitoring various electrodes. The combined use of nitrate and oxygen electrodes for measuring the activity of *Pseudomonas aeruginosa* was described by van Kessel [4]. However the system required separate meters and recorders for each electrode, which is costly both in terms of space and money, and would also not permit the convenient changing of the types of electrode in use. This paper describes the modification of van Kessel's original equipment for easy use with various combinations of electrodes, and the use of a microcomputer both to control the reading of redox and ion electrodes via a single meter, and as an electronic multi-point recorder.

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Materials and Methods

Hardware

The microcomputer was a BBC B (Acorn, OS 1.2) fitted with BASIC 2, the Acorn Disc Filing System and the Watford Dumpout ROM. Interaction with the electrodes was via an interface (type 532.001) manufactured by UNILAB (U.K.). The redox and ion electrodes were plugged into the computer-controlled switches on the interface. The output from the switches was connected via a single plug to the electrode socket on the meter, and the recorder output from the meter to the first of the analogue input ports on the interface. When electrodes with independent amplification were involved, they were connected to the other three analogue ports. The electrodes used with this system came from various manufacturers and included oxygen (Yellow Springs Instruments Co., U.S.A.), nitrate, redox and their calomel reference (Radiometer, Denmark), and ammonia (Orion Research, U.S.A.). As will be seen, the diameter of the electrode is almost as important as its sensitivity. For amplification of the electrode signals, where necessary, Radiometer (Copenhagen) PHM85 and Corning-Eel digital research meters have been used in this work, but any meter with a high impedance input which is suitable for use with ion electrodes and which has an output for a recorder would be suitable. The program has been written for use with the Gemini Star printer but will also function with most Epson printers or the Brother HR5. Only one line needs to be changed to allow the use of another screen dump ROM, or alternatively to call a printing procedure.

Because each electrode has different storage requirements when not in use, it has proved most convenient to mount each electrode in an individual holder. This obviates the necessity for removing the electrodes from the holder at the end of an experiment, and reduces the chance of damaging them. Each holder makes up a quarter of a cylinder so that any four can be combined for an experiment. Should fewer than four electrodes be required, solid quarter cylinders can be used to replace superfluous electrode holders. As the reference electrode was most likely to be used at all times, a small hole was drilled through its holder to allow both the escape of air from the test vessel and the addition of solutions to the experimental mixture once the electrodes were in place. The holder for the oxygen electrode was also modified by the provision of two tubes which would allow the test mixture to be pumped rapidly out of the test vessel, through a sparging chamber (e.g., for extra oxygen or nitrous oxide) and back into the test vessel again. For use, four holders are slotted together to form a cylinder (with a small perspex block in the centre to prevent slipping) and held firmly by two O-rings.

Experiments are done in a round, straight-sided glass vessel in which the electrode cylinder fits precisely. The working volume in the vessel currently in use is 30 ml. A constant temperature is maintained by means of a water jacket. The whole unit is mounted on a magnetic stirrer.

Software

Flow charts for the program are shown in Fig. 1A and B. In addition to providing information for the running of the program, the start-up procedure allows time for the electrodes to adjust to the temperature and ionic levels in the sample. The internal

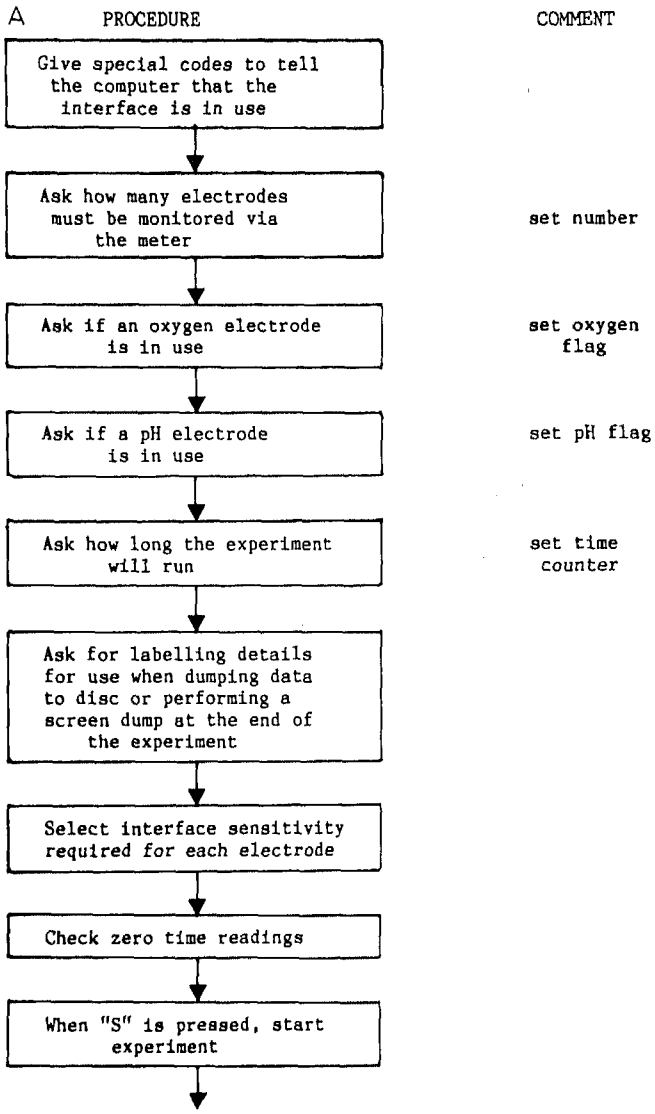

Fig. 1A. Flow sheet for the start-up procedure in the program.

clock of the computer is used in the plotting of the data and to control the length of the experiment. The graphics MODE 1 of the BBC is used for the screen display during the experiment as this allows high resolution graphics in four colours (the background and three others). By using dotted and solid lines, the lines from six electrodes can easily be displayed at any one time. If more lines were required, the sixteen colours of the BBC's MODE 2 could be used but this would mean sacrificing resolution. The oxygen and pH readings are handled separately as these electrode

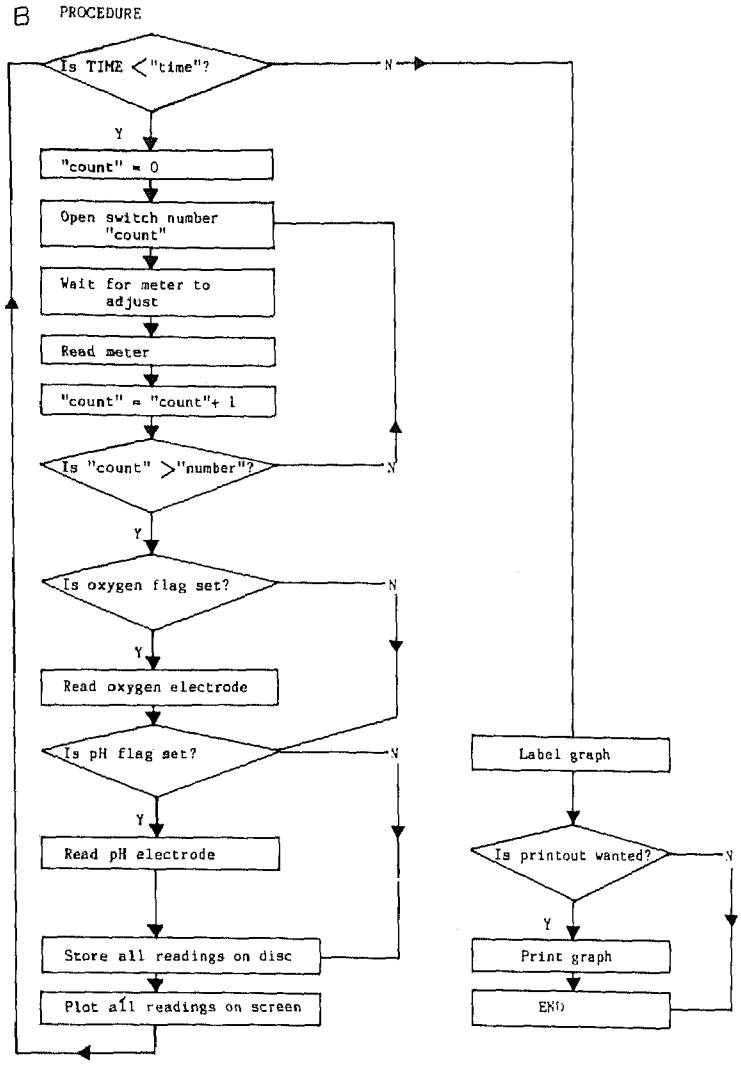

Fig. 1B. Flow sheet for the experimental section of the program.

signals are individually amplified. The program has been written using the facility in BBC Basic for the use of Procedures within a structured programme. This means that the addition of a new electrode to the system only requires the addition of its name to the start-up menu and the addition of a Procedure to handle any special sensitivity or plotting requirements. A print-out of the program is available on request, together with a short program for recovering the data from disc. A program which will allow the calculation of user-determined areas of slope on the screen is under development.

Miscellaneous methods

Both *Thiosphaera pantotropha* and the media used have already been described [1]. The cells used for the sample experiment shown in the results section were grown in an acetate-limited chemostat with ammonium as the sole nitrogen source, and with the dissolved oxygen controlled at 30% of air saturation at 37 °C. The culture had not been exposed to nitrate before the start of the experiment. Acetate was added to the 30 ml sample at the start of the experiment, and nitrate was then added at the indicated intervals.

Results and Discussion

Figure 2 shows a sample printout from an experiment combining nitrate, oxygen and redox electrodes. Previous attempts to show that oxygen and nitrate can be simultaneously used by this organism had involved the measurement of oxygen utilization in one suspension while nitrate reduction was measured in a duplicate. The computer/electrode combination has made it possible to do the whole experiment in a much more direct way. Also, before cultures could be screened for denitrifying ability it was previously necessary to set up a respirometry experiment, and the physiological state of the test organisms could very well change during the

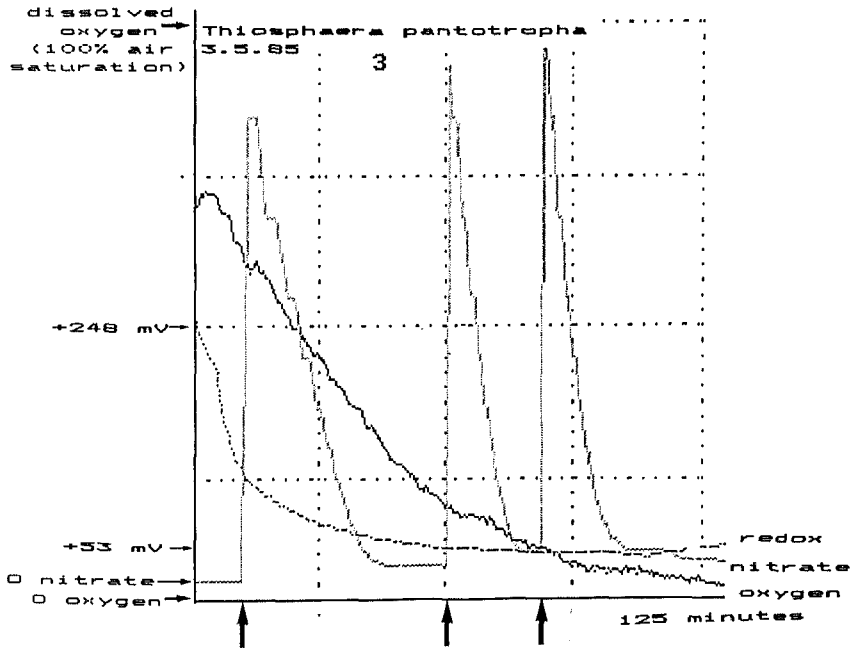

Fig. 2. Sample printout from an experiment where the use of oxygen and nitrate by aerobically grown *Thiosphaera pantotropha*, together with redox changes in the culture, were monitored. The arrows indicate the addition of nitrate (50 μM). Redox readings were corrected for the reference electrode after calibration with quinhydrone solutions at different pH values.

45 min gassing period needed to make the equipment anaerobic for the measurement of nitrogen production. With the new system, cultures can be sampled and tested immediately.

This technique has now successfully been used for the screening of new isolates for constitutive nitrate reducing systems, and is currently in use for the monitoring of heterotrophic nitrification/denitrification by *T. pantotropha*. The method is fully adaptable, and could be used for the monitoring of any metabolite for which there is a suitable electrode available. The main limitation lies in the requirements of individual electrodes. For example, the nitrite electrode available from Orion can only be used below pH 2.0 and could not therefore be directly combined with, for example, the ammonia electrode from the same manufacturers as this requires alkaline conditions. It has proved possible to read nitrite in these experiments only by fitting the electrode with a flow-through cell into which test culture and an acid solution are slowly pumped through converging tubes. This necessitates the use of duplicate cultures – one for the other electrodes and one for nitrite. Again, although the use of the ammonia electrode with living organisms is limited to those cells which are active above pH 9.0, it can be very useful in combination with the oxygen electrode for the measurement of nitrifying enzymes in cell extracts, an assay normally done at pH 10.0.

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References

1 Robertson, L.A. and Kuenen, J.G. (1983) *Thiosphaera pantotropha* gen. nov., sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. J. Gen. Microbiol. 129, 2847 – 2855.

2 Robertson, L.A. and Kuenen, J.G. (1984) Aerobic denitrification: a controversy revived. Arch. Microbiol. 139, 351 – 354.

3 Robertson, L.A. and Kuenen, J.G. (1984) Aerobic denitrification – old wine in new bottles? Antonie van Leeuwenhoek 50, 525 – 544.

4 van Kessel, J.F. (1975) A simple respirometer for measuring oxygen and nitrate consumption in bacterial cultures. Water Res. 9, 417 – 419.