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Technical Note

A TURBIDOSTAT DRIVEN AND CONTROLLED BY MICROCOMPUTER

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ABSTRACT

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An algal culture system is described which is regulated as a turbidostat. The system allows automatic sampling and recording of variables such as chlorophyll, biomass, pH and temperature. In addition to following the growth of the culture, it is possible to stabilise the culture to produce a constant predetermined concentration of algae.

INTRODUCTION

In the continuous culture of micro-organisms (bacteria, phytoplankton) growth takes place under steady-state conditions; that is, growth occurs at a constant rate and in a constant environment (Monod, 1950; Herbert et al., 1956). Chemostats, where the population level is controlled by dilution rate, have been used by many authors to study the relation between growth rate and nutrient concentrations (Paasche, 1973; Droop, 1974). More recently continuous culture using a turbidostat has been described (Falowski, 1984; Post et al., 1984). In this case it is the biomass level which is monitored to dilute the culture. We built such a system to feed appendicularians in culture (Fenaux and Gorsky, 1979) in our laboratory.

DESCRIPTION OF TURBIDOSTAT

The system (schematically outlined in Fig. 1) consists of an incubator with an aerator and magnetic stirrer, a flask of nutrient medium, a multichannel peristaltic pump, three solenoid valves, a Turner 111 fluorimeter, and pH and temperature sensors. The fluorimeter, pump, sensors and valves are connected with the computer.

The centre of the system is an Apple IIe microcomputer with 64 K storage capacity, linked to a dual disk drive, an Epson MX 80 printer and a monitor screen. An ADALAB card (Interactive Microware Inc.) is used as interface between the Apple and the sensors. This card is programmed

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in machine language which accesses to the following inputs and outputs using instructions in simple Basic:
— real-time clock.
— elapsed time counter.
— analog-digital input and digital-analog output.
— serial and parallel input and output sockets.
Real time is thus always available on the monitor screen; precise delays can be obtained; analog values can be acquired and peripherals are under command via the two input—output sockets. Since the ADALAB card has only a single analog-digital input, an 8-way multiplexer (HEF 4051B) was added; selection of one of the 8 ways is programmable via the parallel output socket of the card.

The use of the multiplexer enables connection of 8 analog sensors. At present we use 3 of the 8 inputs which are connected to a temperature sensor, a pH meter and a fluorimeter. In vivo fluorescence appeared to be a good measure of the algal biomass for the species tested.

The analog-digital inputs of the ADALAB card accept voltages in the following ranges: − 0.5 to + 0.5 volts; − 1.0 to + 1.0 volts; − 2.0 to + 2.0 volts; − 4.0 to + 4.0 volts, each with 12-bit resolution. It is therefore necessary to choose one of these ranges and to amplify or attenuate the output voltages of each sensor appropriately.

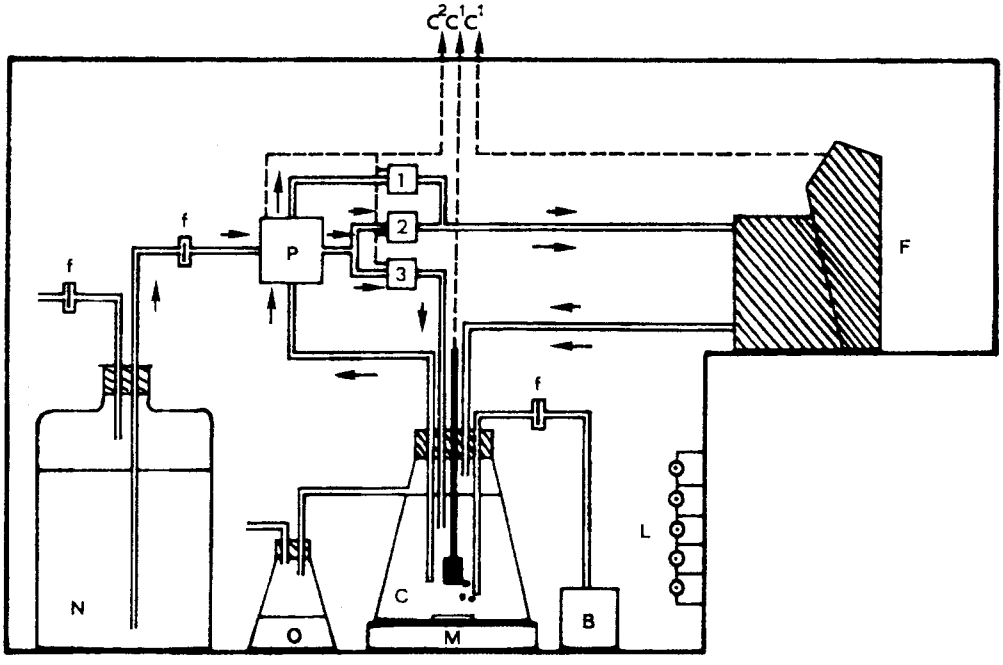

Fig. 1. General description of the turbidostat. B, aerator; C, culture flask; C1, connections between computer and sensors; C2, connections between computer, pump and solenoid valves; 1, 2, 3, solenoid valves; F, fluorimeter; f, Whatmann GF/C filter; L, light source; M, magnetic stirrer; N, nutrient medium; O, overflow flask; P, pump.
→ nutrient; ——► culture.

The temperature sensor is a semi-conductor sensor (Analog Devices AC 2626-K4) which delivers 300 μA at 20°C and 1 μA/°C with near-perfect linearity. The current was fed into a current-to-voltage converter.

The pH sensor is a Knick 646 pH meter with a combined Ingold electrode giving 1 mV for 0.01 pH unit (700 mV at pH 7.0). The fluorimeter output which is between 0 and 10 mV (according to the in vivo fluorescence of the culture) is amplified prior to the ADALAB card input.

The parallel input—output sockets of the card controlled the opening and closing of the solenoid valves (24 V D.C.), and switched the pump on and off via electronic relays (220 V A.C).

OPERATION OF TURBIDOSTAT

At the start, the culture flask contains the culture medium (sea water filtered through a 0.22 μm filter enriched with 1% ES Provasoli medium, or Guillard and Ryther medium) and the chosen phytoplankton inoculum. All the valves (SV) are closed and the pump inactivated. At regular 30-min intervals, the " data collection" cycle is activated, i.e.

1 — opening SV 1 between the culture and the fluorimeter.
2 — starting the pump for the fluorimeter circuit.
3 — a delay to refill the fluorimeter cuvette.
4 — collection of data: fluorescence, temperature and pH.
5 — closing SV 1.
6 — opening SV 2 between the nutrient medium to rinse the system and the fluorimeter.
7 — a delay for rinsing.
8 — closing SV 2.
9 — stopping the pump for the fluorimeter circuit.

At the end of the "data collection" cycle, the fluorescence level is measured. If a maximal preset value is attained or exceeded, the "enrichment/dilution" cycle is started, i.e.

1 — calculation of the volume to be withdrawn to obtain the "plateau" level desired.
2 — opening SV 3 between the nutrient medium and the culture flask.
3 — starting the pump in the nutrient medium circuit for the time required according to the calculation in step 1.
4 — closing SV 3.
5 — stopping the pump in the nutrient medium circuit.

On the monitor screen the program shows continually in text mode: date, hour, means and standard deviations (of 20 sampled values taken at 50 ms intervals) of each of the three variables monitored (fluorescence, temperature and pH); and in graphic mode: fluorescence as a function of time.

At each data collection, information in the text mode is printed and

recorded on the diskette. If there is an interruption in mains supply, the program is automatically re-initiated without loss of data and the screen shows the graph of fluorescence.

RESULTS

As an example, the growth and stabilization of a culture of *Hymenomonas elongata* Droop (Braarud) was followed in the turbidostat maintained at 17 ± 0.5°C under constant illumination of 250 $\mu E\ m^{-2}\ s^{-1}$. The inoculum consisted of $1 * 10^4$ cells ml^{-1}, and f/2 from Guillard and Ryther (1962) was used as culture medium. The culture was stabilised at a fluorescence value of 8 mV, corresponding to a cell number of $1.5 * 10^5$, after about 75 h. Once or twice a day, cell number was counted in eight columns (8 * 1 mm) of a hemocytometer.

It appears from Fig. 2 that with the present turbidostat, algal cell concentrations can be kept constant at a preset concentration (PC) with an accurate adjustment: PC = 8 ; X = 8.077 ; s = $3.367 * 10^{-2}$; n = 170.

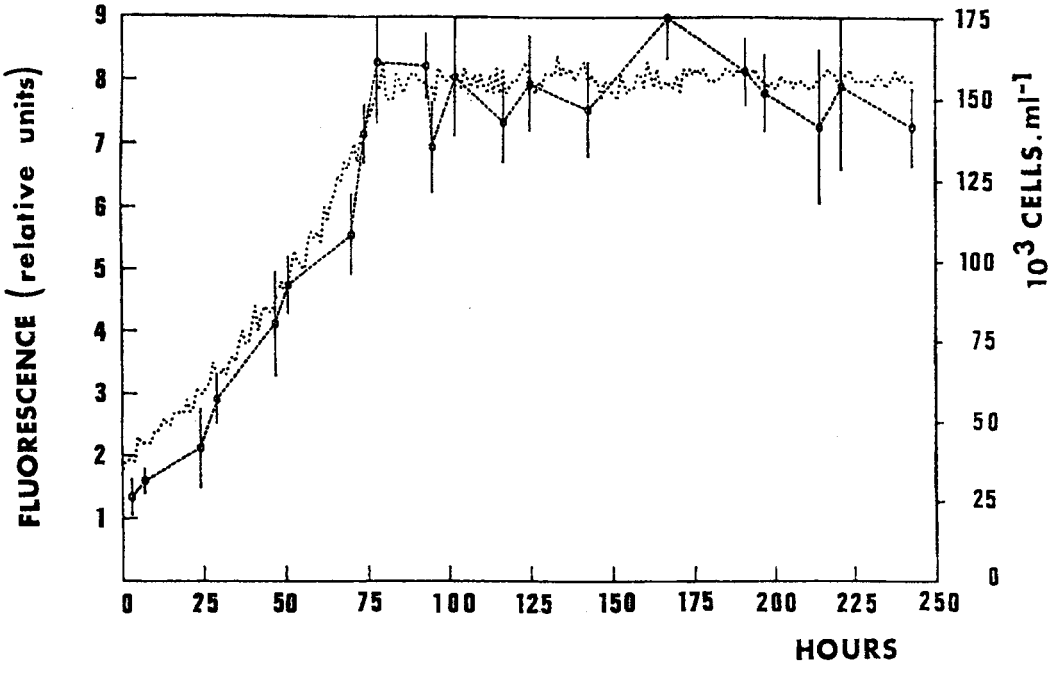

Fig. 2. Growth and stabilisation of a culture of *Hymenomonas elongata*. Dotted line: fluorescence readings (one measurement per hour). Dashed line: average and standard deviation from hemocytometer counts.

This precise adjustment, coupled with the computer screen display of fluorescence as a function of time and the automatic re-initiation of the program without loss of data after interruption in mains supply, makes the system accurate, easy to use and safe.

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