Engineering Experiment Station
Reprint No. 1009

Reprinted from ENVIRONMENTAL Science & Technology
Vol. 1, June 1967, Pages 503–505

Apparatus for Automatic pH Control in Algal Cultures

Fred V. Soltero[1] and G. Fred Lee**
Water Chemistry Laboratory, University of Wisconsin, Madison, Wis. 53706

■ An electronic pH controller has been designed and tested for the control of pH in algal cultures. The output of a laboratory pH meter is used to control the addition of a CO_2-air mixture to the culture. The apparatus controlled the pH of 7, 8, and 9 of cultures of *Scenedesmus quadricauda* to ± 0.1 pH unit.

Various investigators (Gerloff, Fitzgerald, *et al.*, 1950; Osterlind, 1949; Zehnder and Gorham, 1960) have used different ways to control the pH of an algal culture in order to determine the optimum growth under specific experimental conditions. These methods include manual addition of acid or base to the culture medium at constant time intervals, addition of large amounts of pH buffers, such as Tris, aeration with CO_2-enriched air at different partial pressures, and the use of chemical species that, upon utilization, tend to counteract the normal pH changes in solution. In general, these procedures either do not provide precise pH control or require the addition of large amounts of compounds to the medium. This paper describes the construction and operation of a relatively inexpensive apparatus that will provide precise pH control in algal cultures.

Experimental Methods and Materials

Figure 1 is a schematic diagram of the culture apparatus used in this investigation. The pH meter was a Beckman Zeromatic pH meter. A combination glass-calomel electrode was inserted into the culture vessel, which was a 4-liter borosilicate bottle with two perforations on its shoulder. The culture vessel was placed on a wooden frame above a

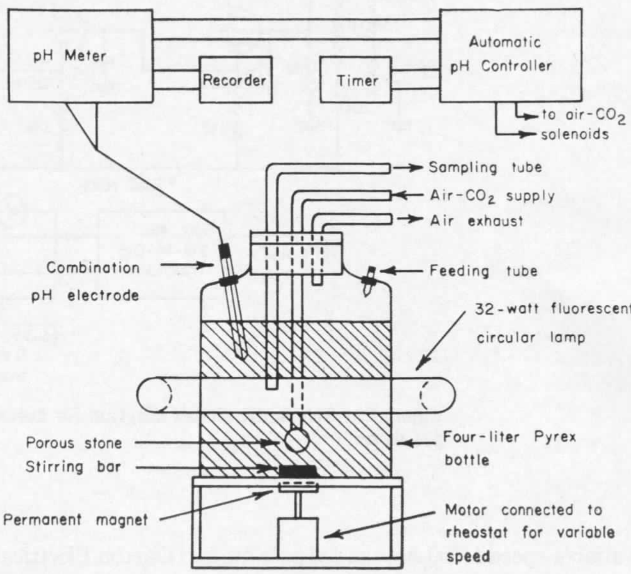

Figure 1. Schematic diagram of culture system with automatic pH control

[1] Present address, College of Agriculture and Mechanic Arts, University of Puerto Rico, Mayaguez, Puerto Rico.

**Currently: G. Fred Lee & Associates, El Macero, CA
gfredlee33@gmail.com - www.gfredlee.com

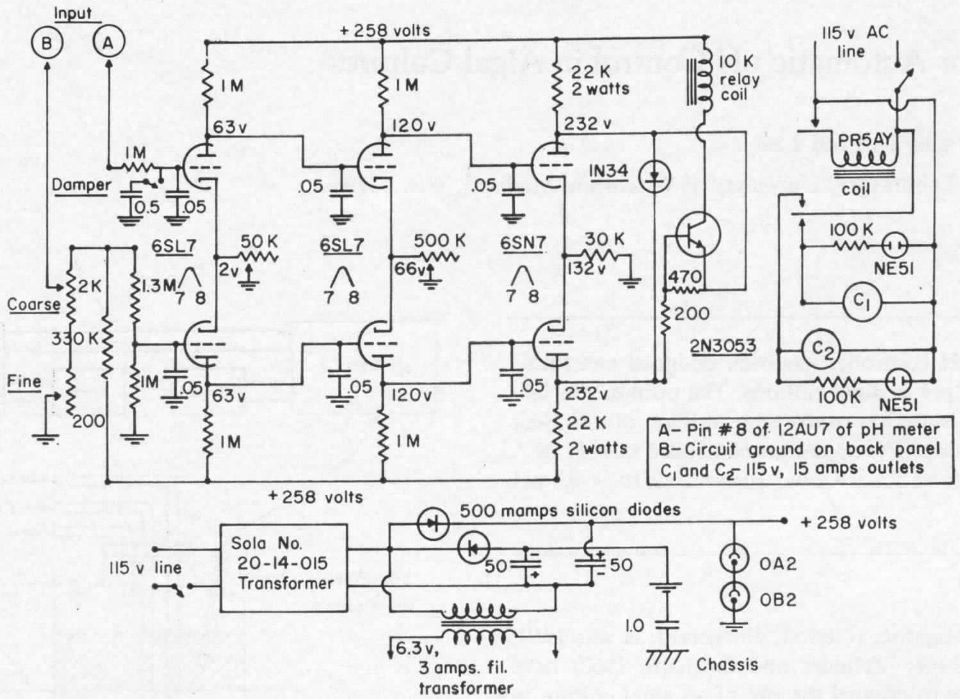

Figure 2. Schematic circuit diagram for automatic pH controller used with the Beckman Zeromatic pH meter

variable-speed 1/100-hp. shaded pole motor (Dayton Electrical Manufacturing Co., Chicago, Ill.) with a permanent magnet attached to the shaft. The rate of stirring was controlled by a Variac transformer. The culture was illuminated with a circular 32-watt fluorescent lamp placed around the middle of the bottle. The culture vessel was stoppered with a solid rubber stopper through which three glass tubes were inserted; one extended to the bottom of the vessel with a diffuser stone attached for the CO_2-air supply, another tube extended about halfway down from the top of the liquid and was used for sampling, and the third short tube served as the escape route for the gas mixture to the atmosphere. The CO_2-air mixture was passed through a sterile cotton filter to remove any contaminants.

A Rustrak recorder, 100-μa. full scale, was connected to the pH meter to provide a record of culture pH.

The automatic pH controller was an electronic device constructed in this laboratory to control the solenoid on the CO_2-air supply line. Figure 2 is a schematic circuit diagram of the automatic pH controller. The input signal for the automatic controller was taken from the cathode of the output tube of the pH meter. Initially, the system was balanced at the desired set value. Under these conditions, no output voltage difference was obtained from the two channels of the d.c. amplifiers on the controller. When there was a slight difference in the pH value from the preset value, an output voltage was obtained from the amplifiers. The voltage caused a transistor to turn on the current through the relay which opened or closed the solenoid valve installed in the CO_2-air supply lines. Thus, whenever algal utilization of CO_2 resulted in an increase in medium pH, the controller opened the CO_2-air line and the gas mixture bubbled through the medium until the pH was restored to its original value. An interval timer was attached to the controller to register the total open time of the solenoid.

The CO_2-air mixture was fed at a rate of approximately 1400 cc. per minute measured at 1 atm. and 25° C. At pH values less than 9, 5% CO_2 in air, by volume, was found to be satisfactory. At pH 9, 2% CO_2 in air was used. The maximum variation of pH in the culture medium was ± 0.1 pH unit.

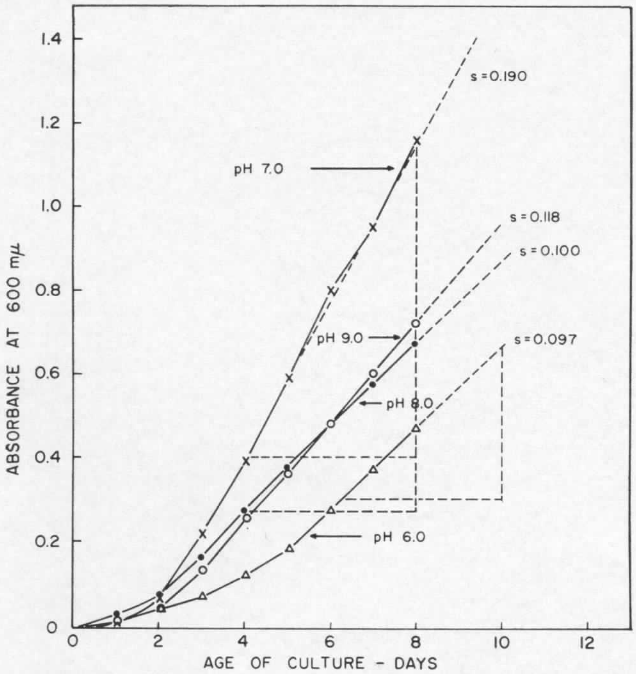

Figure 3. Growth curves of *Scenedesmus quadricauda* in Gorham's medium with automatic pH control

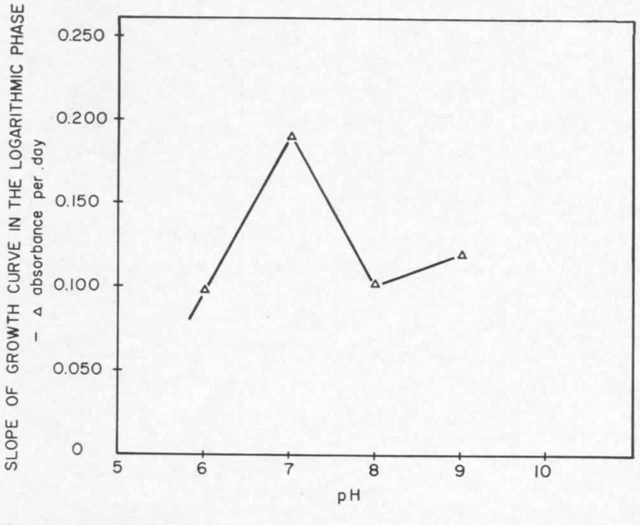

Figure 4. Effect of pH on growth of *Scenedesmus quadricauda* in Gorham's medium

The design of the controller is a modified version of a circuit provided by M. J. Johnson, University of Wisconsin, Department of Biochemistry. The total cost of the controller was about $100.

A unialgal culture of *Scenedesmus quadricauda* (Indiana Culture Collection No. 77) was used to test the automatic controller. All external connections of the culture vessel were plugged with cotton and the unit was autoclaved for 30 minutes at 15 p.s.i. Gorham's medium (Hughes, Gorham, *et al.*, 1958) was used in all experiments and the temperature of the culture medium was maintained at 23° C.

Growth in the cultures was determined by an increase in absorbance of the solution measured at 600 mμ in a Bausch and Lomb Spectronic 20 spectrophotometer. An absorbance of 1.86 yielded 1.45 grams per liter of dry weight.

Results and Discussion

Figure 3 presents the growth curves for *Scenedesmus quadricauda* in Gorham's medium at pH values of 6.0, 7.0, 8.0, and 9.0. Examination of this figure shows that 2 to 5 days after initiation of the run, the cultures entered into an apparent log growth phase. During the log growth phase, the demand for the CO_2-air mixture was fairly frequent. At pH 8.0, the automatic controller fed CO_2-air mixture at approximately 10-minute intervals. The maximum deviation from the preset pH value during the run was ± 0.1 unit. The slopes of the growth curves during log phase can be used as an index of the effect of pH on growth. These slopes are plotted against pH in Figure 4. The apparent optimum growth for the organism in the pH range 6 to 9 is at pH 7. Further studies are needed to verify these results, since only single runs were made at each pH. These results demonstrate the applicability and reliability of the pH controller for studies on the growth of algae at constant pH.

Literature Cited

Gerloff, G. C., Fitzgerald, G. P., Skoog, F., "The Isolation, Purification, and Nutrient Solution Requirements of Blue-Green Algae," Symposium on the Culturing of Algae, pp. 27–44, Charles F. Kettering Foundation, Dayton, Ohio, 1950.

Hughes, E. O., Gorham, P. R., Zehnder, A., *Can. J. Microbiol.* **4,** 225–36 (1958).

Osterlind, S., *Symbolae Botan. Uppsalensis* **10,** 10–31 (1949).

Zehnder, A., Gorham, P. R., *Can. J. Microbiol.* **6,** 645–60 (1960).

Received for review March 8, 1967. Accepted May 18, 1967. Investigation supported by Training Grant 5T1-WP-22-04 from the Federal Water Pollution Control Administration. Support also given by the University of Wisconsin Engineering Experiment Station and the Department of Civil Engineering.