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Computerized Bioassays: Two Examples

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In the last years quite a few approaches to bioassays were proposed by several investigators, because chemical analytical techniques have not yet been developed to cover the full range of possible water pollutants. Especially algal assays continue to be used extensively in toxicity testing. But still there are only few existing assays which are standardized. The high variability and different sensitivity of organisms require a great amount of measurements and statistical data interpretation.
To minimize variances in the test results and to faciliate a statistical estimation, bioassays should be connected to a data system. In the following, two bioassays for algizide substances (algae-fluorescence bioassay, Benecke et al. 1982), Phormidium inhibition test (Sayk and Schmidt 1983) are presented, which run nearly completely controlled by a microcomputer system (Commodore 8032).

MATERIALS AND METHODS

A description of the test principles has been given elsewhere (Schmidt, 1983). The fluorescence test is based on the measurement of the spontaneous, variable fluorescence as one parameter of a plant's physiological state. Normally, the electron-transport in the photosystems I and II is then undisturbed when a low fluorescence is indicated. A change of the fluorescence emission due to substances which affect or block photosysnthesis, is well known since Kautsky (1943). Algal fluorescence normally shows very distinctive effects after a toxicant dosage (Franck et al. 1969; Metzner 1969; Arndt 1972; Sellner 1982). For the test, unicellular green algae are used (Scenedesmus subspicatus, Chlorella fusca). To obtain a fluorescence signal, the algae are pumped into a flow-through cell and then exposed to light (0.5 sec.). The emitted fluorescence is measured at 685 nm (+/- 5 nm) by use of a photomultiplier. The microcomputer has three main functions (Fig. 1):

1. Process control
2. Recording
3. Data storage and mathematical evaluation

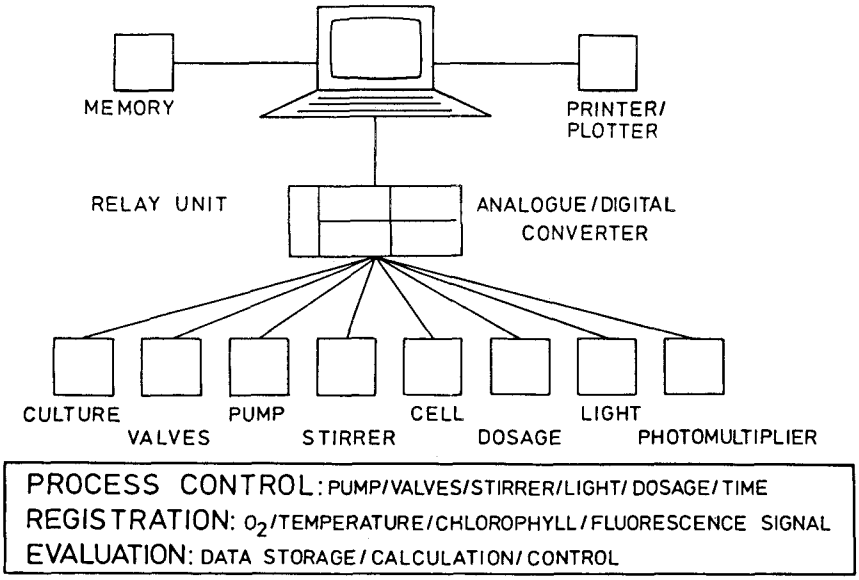


Figure 1. Scheme of the microcomputer functions

The actual standard of this test-device allows a complete system control by the micocomputer. After the selection of the test-parameters the assay is started, performed and repeated automatically by the computer. Fig. 2 shows a flow chart of the computer functions. The software is written in BASIC using several ASSEMBLER subroutines. The program begins with some routine questions (e.g. algae species, date etc.). The chlorophyll content of the algae culture, pH and O_2 are measured every 10 min. An analogue/digital converter enables the data transfer from the test-system to the computer. The computer is also connected to a micro-proportioning pump for toxicant dosages. A test-run starts with the measurement and mathematical evaluation of non-affected fluorescence curves. The data are then stored and compared. A toxicant dosing then begins, when the results of the actual measurements (depending on the actual chlorophyll content of the algae) do not deviate by more than 5%. The chlorophyll content of the algae culture normally varies between 850 and 900 µg/l. During the registration of a toxicants influence, the pumping of algae is stopped. Algal fluorescence is registrated every 10 min. Fig. 3 shows the evaluation criteria of a fluorescence curve. If one of the 5 parameters exceeds or underceeds the values of the "normal" fluorescence, the toxicant will then be classified as toxic for algae.
Fluorescence curves and evaluations can be displayed and printed as graphs. The test allows an evaluation of a toxicants sublethal influence on algae within 30-60 min. The test is in revision for a continous classification of surface or sewage water.

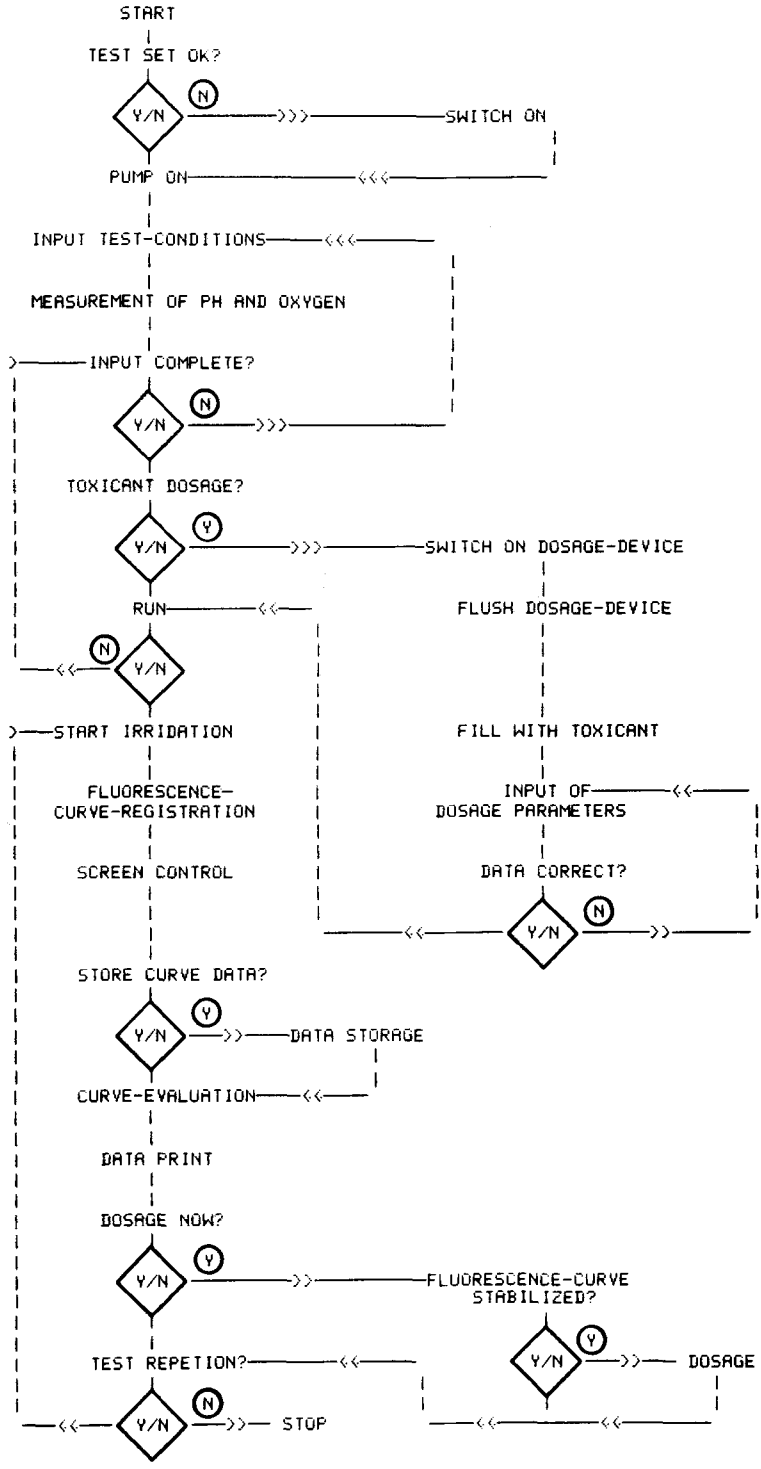


Figure 2. Flow chart of the fluorescence bioassay

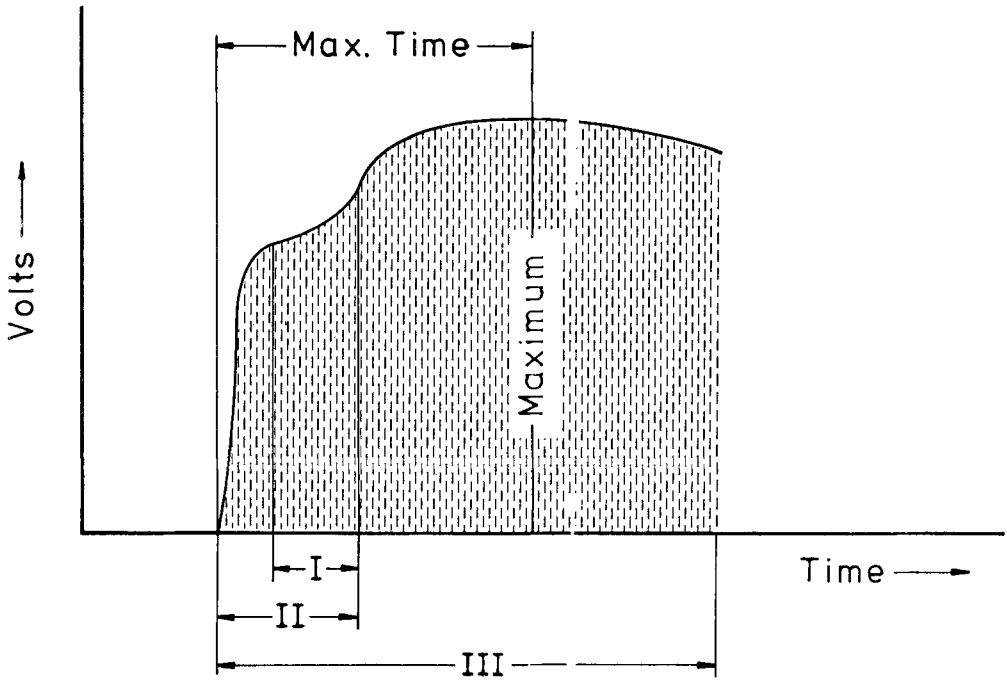
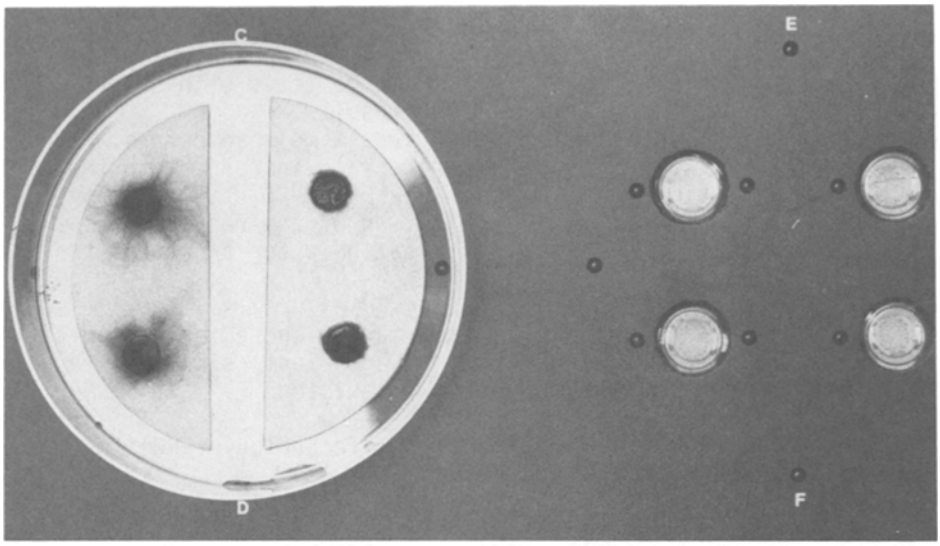


Figure 3. Normal fluorescence curve and evaluation criteria: Maximum, Maximum time, Integral I, II, III.

The Phormidium inhibition test is based on the creeping ability of the blue alga Phormidium autumnale. Toxicants normally inhibit the creeping (Noll and Bauer 1973; Benecke 1980; Sayk and Schmidt 1983). To perform the test, about 1 g of Phormidium algae, which can easily be kept in culture, are crushed in 50 ml nutrient solution and filtered through a 5 µ membrane filter. Circular pieces of this membrane filter (5mm) are placed on filter paper, which covers a pair of photodiodes (Fig. 4a; pair C and D can be seen). 8 pairs of photodiodes can be compared, each measuring normal creeping algae and an algae-spot under the influence of a toxicant. Fig. 4b shows the measuring device with 16 photodiodes. The effect of a particular toxicant is evaluated by the registration of the different darkening of the photodiodes, e.g. their rising resistance. The tolerances in the different photodiode fabrications can be compensated by electronical balancing. Using 16 photodiodes, 8 different concentrations of a toxicant can be measured at the same time. The measuring system is connected to a microcomputer, which records the mV-output of the photocells (A/D-converter). The results are stored and then compared with previous results. An algae toxic influence is noticed at a 20% deviation level. A creeping stimulation can also be measured in the same manner. The measurung device including the photodiodes is produced by U KNAPP, Schwerte, FRG at a price of $2500.



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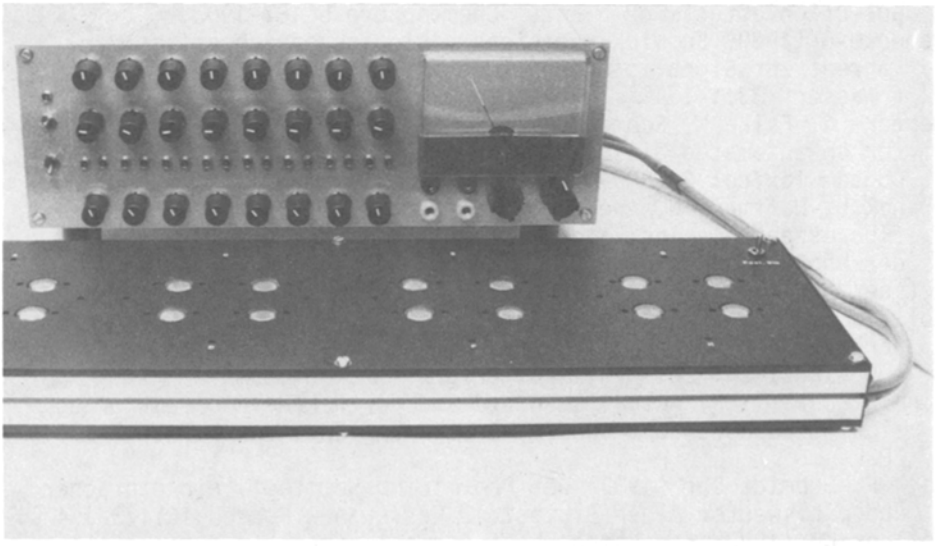

4b

Figure 4a. Phormidium spots on filter-paper covering photodiodes Two pairs of photodiodes (C and D) can be seen. On the left normal creeping algae, on the right contaminated algae.

Figure 4b. Measuring device for the Phormidium bioassay. On the scale the mV-output of each photodiode can be read. Electronic comparators balance different zero-deviations of the photodiodes.

RESULTS AND DISCUSSION

The main advantages of the connection bioassay-microcomputer can be described as following:

- The tests can be performed without any disturbance or influence by the experimenter. The computer runs the tests with exact repetitions and produces "objective" results.
- The experiments can be performed in less time than before. A considerable number of test runs are necessary in respect to the biological material.
- The computer supports a quick evaluation of the results.
- Computerizing bioassays may be of great help for a better standard of assays.

It is possible, that one technican can manage these tests from a central control station (water work, sewage plant) similar to a water quality monitoring with fish by television.

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