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A Technique for the Enumeration of Unicellular Algae

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The algal assay bottle test (AABT, Miller, 1978) using Selenastrum capricornutum Printz is recommended as a model test system for evaluating the influence of chemicals on algal growth. Similar tests have been also proposed by the Organization for Economic Cooperation and Development (OECD 1981) and the American Public Health Association (APHA 1980). An algal toxicity test method is currently under review by the American Society for Testing and Materials (ASTM). These methodologies have suggested that electronic counting techniques be considered.

Direct methods of algal growth analysis include microscopic cell enumeration using a hemacytometer counting chamber and gravimetric measurements of algal cell dry weight. These are tedious, time consuming, and subject to errors that may exceed ±10% (Bartsch 1971, Miller 1978).

Algae investigators have also utilized the Coulter blood cell counter in combination with a (MCV) mean cell volume computer accessory (Stein 1975, Fitzgerald 1975, Shoaf 1976, Roline 1979, Rehnberg 1982, Canton, 1983). However, little or no information is available on the preparation of algal suspensions for electronic counting, nor are the advantages or limitations of electronic instruments considered.

Algal cell counts have been reported using an industrial Coulter electronic counter, model TAII (Dixon, 1979). However little methodology information is available which is needed to enhance accuracy, insure reproducibility, and to promote standardization of algal cell size analysis.

This model counter is used extensively in industry, from quality control analyses to research investigations and is recommended in ASTM test protocols for particle size measurements of airborne particulates (Allen 1981).

This instrument is particularly well suited for systems having a wide particle size distribution with diameters from 0.6 to 224 um. This unit operates with nine different aperture tubes ranging from 30 to 560 um. Suspension sampling performed in the manometer mode provides three convenient manometer volumes: 0.05, 0.5, and 2.0 ml. In contrast, the Coulter Blood Cell Counter, which is used for routine and research hematology, is supplied only with 70 and 100 um aperture tubes (Coulter Electronics, Inc., 1982).

The purpose of this study was to develop a reliable technique using the Coulter electronic counter TAII for algal testing. The advantages and limitations in using such electronic counters are discussed.

MATERIALS AND METHODS

The Algal Assay Bottle Test (AABT: Miller, 1978) technique was utilized to conduct two consecutive batch tests. The nutrient medium was prepared with 0.45 um filtered deionized water; this nearly particle-free water also provided a final rinse of culture flasks.

An axenic culture of Selenastrum capricornutum obtained from American Type Culture Collection, Rockville, Maryland (ATCC #22662) was continuously maintained in the laboratory by weekly subculturing.

A seven-day-old algal inoculum was used to give a starting optimum cell loading of 2×10^4 cells/ml. This inoculum was transferred into culture flasks using automatic Eppendorf Repeater pipettes equipped with disposable sterile combitips.

Algal growth was measured as cell-count (no./ml) after 4, 7, 10, and 14 days of incubation.

The model TAII Coulter Counter was used in conjunction with a multichannel Coulter Population Counter Accessory (PCA II). The total count, as well as the number of algal cells in each of sixteen size ranges, were determined for a 0.5 ml volume of algal suspension. The actual counting time per sample was usually 25 seconds.

Since the measurable cell size is typically from 2 to 40 percent of the aperture diameter, a 70-um-diameter aperture was selected for analyzing algal samples. This orifice is suitable for measuring Selenastrum cells which range in size from 2.5 to 7.5 um in diameter, 10 to 30 um in length, and 10 to 100 um^3 in volume (Bartsch 1971).

The instrument was calibrated using monosized calibration microspheres having a diameter of 5.58 um.

An algal cell suspension was prepared for electronic counting by transferring one ml of a well mixed algal sample culture to nine ml of an electrolyte solution consisting of one percent sodium chloride. This electrolyte was double-filtered through a 0.22 um filter prior to use.

Selenastrum species is single-celled and usually has no tendency to clump; therefore, there was no need to sonicate or to add dispersing agents prior to counting.

A raw count was defined as the number of algal cells in 0.5 ml of test suspension passing through a precalibrated 70-micron aperture tube. The cell concentration of a sample (no./ml) was determined by multiplying the raw cell count by an appropriate dilution factor.

At numbers above 20,000 raw count, two or more cells may enter the aperture at the same time (coincidence) and, therefore, could be counted as one large cell. A low coincidence level in counting is desirable in order to minimize an artificial decrease in cell-count combined with an increase in cell-volume. A coincidence correction constant using a probability equation relating aperture diameter and sample volume was applied to the experimental count to obtain a corrected count. Generally, when raw counts exceeded 58,000, further dilutions of algal suspension were necessary to allow satisfactory counting. A 5% coincidence level is recommended as a maximum (Coulter Electronics, Inc., 1981, 1982).

RESULTS AND DISCUSSION

The mean values ($\bar{x}$) of algal cell concentration and their associated standard deviations (SD) were calculated and are listed in Table 1 below:

Table 1 Selenastrum Cell Concentration

Incubation Period (Days)	Batch Test I Cell-Count 10^6 cells/ml	Batch Test II Cell-Count 10^6 cell/ml
4	1.16±.12	1.28±.06
7	2.9±.10	2.99±.08
10	3.28±.07	3.21±.05
14	3.21±.17	3.31±.05

Algal cell concentration-mean values and 95% confidence limits are listed in Table 2. These data were calculated utilizing all fourteen algal culture flasks analyzed in the study.

Table 2 Selenastrum Cell Concentration

Incubation Period (Days)	Cell-Count Mean Value 10^6 cells/ml	95% Confidence Limits
4	1.22	1.15-1.29
7	2.95	2.89-3.00
10	3.25	3.21-3.29
14	3.26	3.18-3.34

The results show a high between-sample and between-batch test reproducibility for electronic counting. In these laboratory Selenastrum cultures, cell concentration values were in agreement with earlier algal production studies (Fitzgerald 1975, Miller 1978).

This study illustrates several advantages in analyzing Selenastrum cells with an industrial electronic counter. The use of electronic counters saves cell counting time, and also improves counting precision. More than ten-twelve algal sample analyses could be completed in one man-hour using the Coulter Counter, as compared with four to five analyses using a counting chamber. In contrast with direct counting devices such as the hemacytometer chamber, large numbers of cells are counted in a relatively large volume of algal suspension (routinely 0.5 ml), giving greater precision and statistical reliability (Jelinek 1974, Stein 1975).

The TAII unit provides four convenient sampling modes from which to choose. Sample analyses can be performed on the basis of either preset time, total count, total count per channel, or preset volume using the manometer. The Coulter Counter is the only instrument which directly measures cell volume; all other particle sizing electronic devices measure a cross sectional area or a parameter which depends on area. Since the volume is measured directly, volume distribution can also be determined (Alliet 1972).

A disadvantage of electronic counting is a need for a continuous supply of an electrolyte having a low background particle count. The use of a sub-micron particle filtration system facilitates this important step.

Additionally, the problem of coincidence may lead to a significant artificial decrease in raw cell-count combined with an increase in cell-volume, and may also increase chances of aperture blockage.

Finally, there are some reservations about a particle counting instrument that cannot differentiate between live and dead algal cells (Rehnberg 1982). However, measured dry weights also include the weight of both live and dead algal cells. Determination of the viability of growth-inhibited algae can be achieved by means of subculturing and/or mortal staining coupled with microscopic observation.

In conclusion, there is a need for continued refinement and standardization of algal cell counting using electronic counters, but the advantages of speed and convenience have been clearly demonstrated. Other procedures which relate measurement of some other property to cell concentration, such as chlorophyll fluorescence or changes in optical density, have been used to permit monitoring of Selenastrum cell multiplication (USEPA 1969, Weiss 1971). These measurements of algal growth and productivity should be correlated with an independent measure of biomass, such as dry weight (mg/l) measured or indirectly calculated, using cell count and mean cell volume (MCV) data. In combination with a Coulter Population Counting Accessory (PCA), the model TAII, Coulter Counter can be utilized to determine cell number as well as mean cell volume (MCV um^3) in a single step.

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