# Colorimetric determination of the number of cells in axenic cultures of *Scenedesmus quadricauda* - A comparison with direct counting

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## Abstract

A colorimetric method for determining the number of cells in cultures of *Scenedesmus quadricauda* was compared to direct counting, employing a Fucks Rosenthal haematocytometer.

A stock culture was used to determine the in vivo absorption spectrum in order to quantify the range of wavelengths at which the species peaks occur. Dilution tests were carried out to verify whether the cell suspensions obeyed Beer's law. The growth of this culture was also studied by both colorimetric and direct counting. The results were used to plot a standard curve: optical density units vs number of cells.

A computer program was written to calculate the number of cells at each interval of 0.25% of transmittance or equivalent absorbance units.

## Introduction

The use of colorimetry in the determination of the rate of growth in algae cultures has received much attention because its potential in monitoring growth in continuous cultures (Eppley, 1977) and in bioassays. Therefore we can expect its future routine use in algal culture studies. There is still a need to obtain more accurate measurements with this alternative method (Spencer, 1954; Sorokin & Kraus, 1958; Sorokin, 1973), but its main advantage is that it is very simple, permitting the quantification of several samples in a short period of time.

In order to obtain a good accuracy and reproducibility with this method we must meet some experimental conditions such as: shaking the culture flash before sample collection, use of an adequate spectrometer cuvettes (Harvey, 1953; Mayers, 1952; Spencer, 1954; Sorokin & Kraus, 1958; Sorokin, 1973), and others. On the other hand, as the microscope is not used, the physiological condition of the culture cell cannot be verified (Mayers, 1952).

The present study was inspired on previous work carried out by Sorokin, 1973, and its aim is to verify the correlation between optical measurements (transmittance or absorbance) and the number of cells in axenic cultures of *Scenedesmus quadricauda*.

## Material and methods

The assays were carried out on a stock culture of *Scenedesmus quadricauda* (Strain UFS-030-CHL-023-1) maintained in a Rodhe medium (Rodhe, 1948) in axenic conditions under constant ilumination (3,000 lux) and temperature (20 ± 2 °C).

The *in vivo* absorption spectrum of concentrated cell suspension from the culture was obtained against a sterilized culture medium as a blank. These measurements were done using a Beckman 25 K spectrometer (from 300 to 700 nm), according to Emerson & Lewis (1943). The results are shown in Figure 1.

In addition to these experiments, we performed measurements of the absorbance at three different

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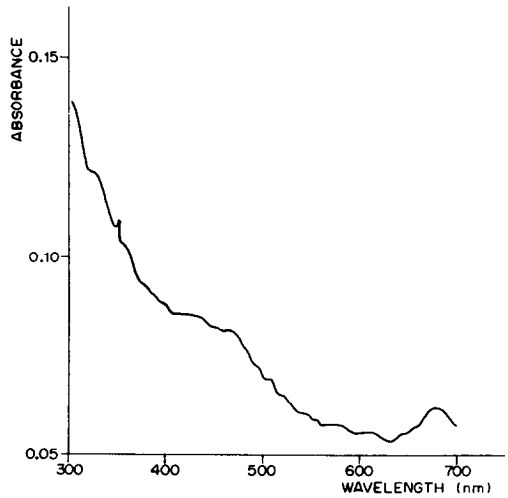


*Fig. 1.* The in vivo absorption spectrum of a cell suspension of *Scenedesmus quadricauda.*

wavelengths: 430, 520 and 660 nm (nominal value of each filter) by using an Atago OE-7 colorimeter. These measurements were carried out by employing a reasonable dense suspension of cells diluted five consecutive times (½, ¼, ⅛, 1/16 and 1/32) as proposed by Sorokin, 1973. The obtained results are presented in Figure 2.

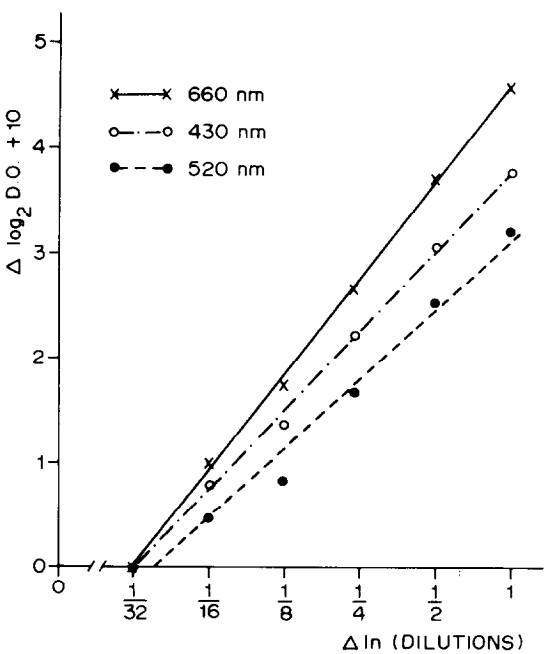


*Fig. 2.* Optical density of the successive dilutions of *Scenedesmus quadricauda* culture determined at 430, 520 and 660 nm.

For comparison, the growth of a *Scenedesmus quadricauda* culture maintained in the same conditions as the stock culture was monitored both by direct counting and the proposed colorimetric method. The countings were performed in a Fucks Rosenthal haematocytometer in which a sample of 3.2 ml was counted (three times). The average was converted to cells $ml^{-1}$ according to Vieira, 1975 (Fig. 3). Colorimetry was carried out using an Atago OE-7 colorimeter (430, 520 and 660 nm). The results are shown in Figure 4.

The conversion factor introduced by Sorokin, 1973 for the *Chlorella sorokiniana* was here adapted for the *Scenedesmus quadricauda.* With this procedure we could establish a standard curve relating optical density to number of cells per unit volume (Fig. 5). Finally a computer program (Fortran IV) was developed to calculate the cell number at each optical density value (Fig. 6).

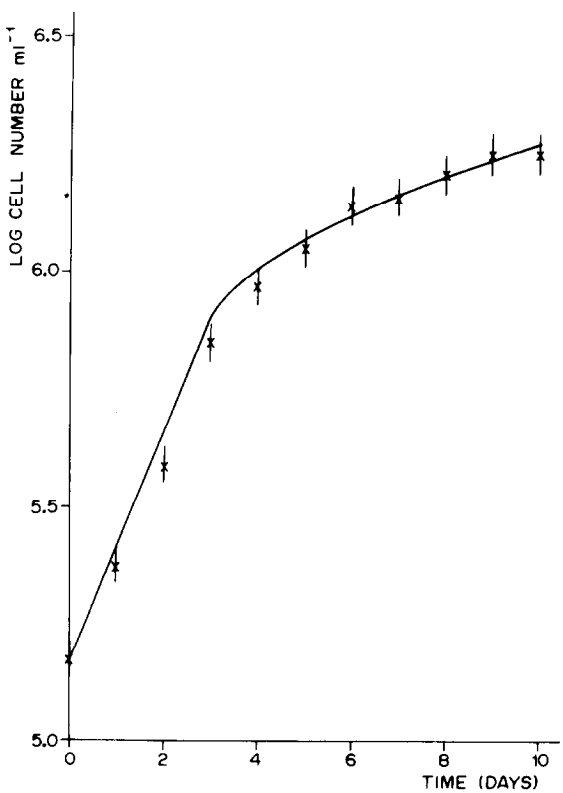


*Fig. 3.* Growth curve of *Scenedesmus quadricauda* obtained with direct counting of cells.

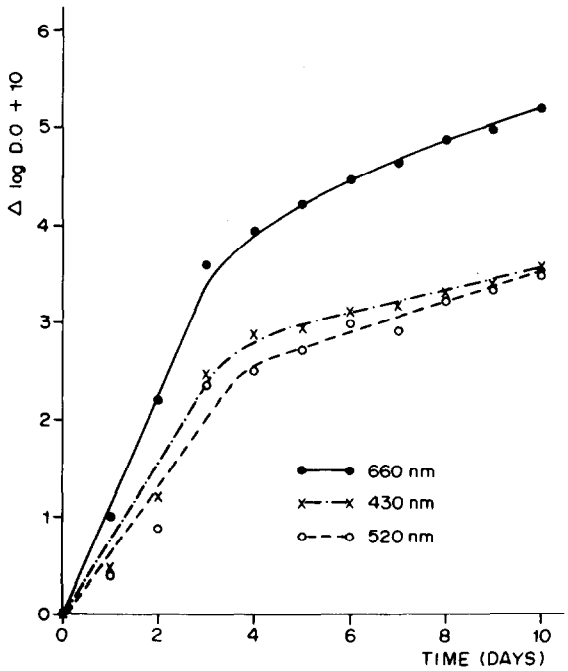


*Fig. 4.* Growth curves of *Scenedesmus quadricauda* culture obtained with the colorimetric method at the indicated wavelengths.

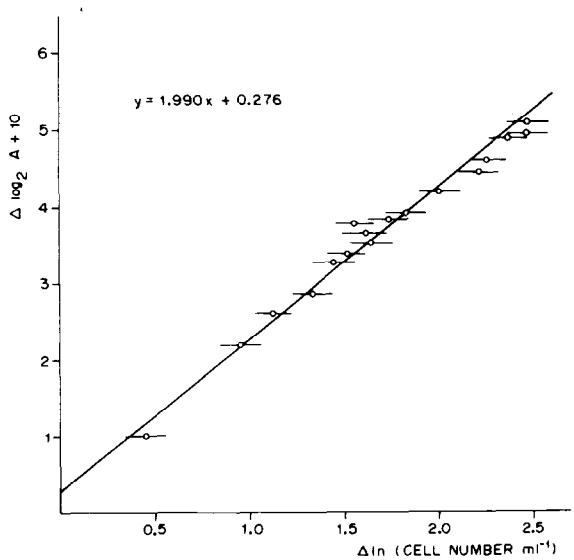


*Fig. 5.* Standard curve relating colorimetric (in the 660 nm wavelength) and direct counting methods.

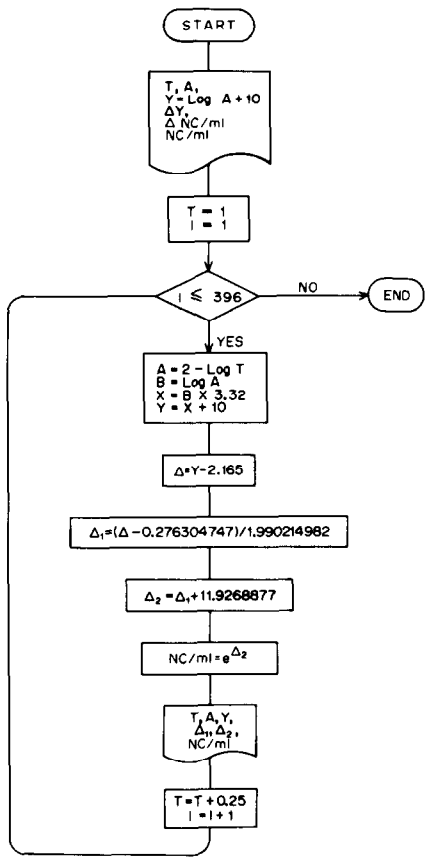


*Fig. 6.* Flow chart of the computer program relating optical measurements with number of cells of *Scenedesmus quadricauda*. The meaning of symbols:

T = transmittance

A = absorbance

NC/ml = number of cells per ml

B = $\log_{10}$ A

X = $\log_2$ A

Y = X + 10

Δ = difference between Y of the observed T (or A) and Y of the T = 99% (or A = 0.044).

1.990 . . . = angular coefficient (relation between Y and the difference between the natural logarithm of the observed number of cells and the natural logarithm of the number of cells equivalent to T = 99% (or A = 0.044).

0.276 . . . = linear coefficient (relation above described).

$\Delta_1$ = result of the convertion of the colorimetric measurement to natural logarithm of NC/ml.

$\Delta_2$ = $\Delta_1$ plus natural logarithm of NC/ml equivalent to T = 99% (or A = 0.044).

i1.926 . . . = natural logarithm of the NC/ml equivalent to T = 99% (A = 0.044).

I = control variable (computational).

## Results and discussion

We can see from Figure 1 that the light absorption by cells of *S. quadricauda* is a function of the composition of the characteristic pigments of the species. This fact is in agreement with earlier results by Steemann-Nielsen (1975). There is high absorption of light in the blue region (400–480 nm), and another peak in the red region (640–700 nm). The lowest values is found arround 600 nm. This fact led us to select the red filter (660 nm). Harvey (1953) and Spencer (1954) working with diatoms and Sorokin (1973) working with *Chlorella* obtained similar results.

The dilution test confirms the choice of the filter and also shows that the culture, as a coloured solution, is obeying Beer's law.

The error of the measurements by the colorimetric method using the 660 nm filter was 8.5%. This error is smaller than that obtained by the use of the blue filter (11.9%) or green filter (34.1%). The error of the method of direct counting is 10%.

The colorimetric method is especially useful for comparative studies. It provides the advantage of minimization of experimental erros. We compared the results shown in Figure 4 (colorimetric method) with those of Fig. 3 (direct counting), which once more confirmed 660 nm as the optimum wavelength relating these two methods.

After the choice of the most adequate filter, a calibration curve (Fig. 5) was obtained from the experimental points of Figure 3 and 4. This curve allows to calculate the number of cells $ml^{-1}$. The computer program quantifies this number of cells for each 0.25% of transmittance unit in a range of 0 to 100% or in equivalent absorbance units (see Fig. 6).

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