Algal density assessed by spectrophotometry: A calibration curve for the unicellular algae *Pseudokirchneriella subcapitata*

Lúcia Helena Ribeiro Rodrigues¹*, Alexandre Arenzon², Maria Teresa Raya-Rodriguez² and Nelson Ferreira Fontoura³

¹Laboratório de Ecotecnologia e Limnologia, Instituto de Pesquisas Hidráulicas, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970, Porto Alegre, RS, Brazil.
²Laboratório de Ecotoxicologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970, Porto Alegre, RS, Brazil.
³Laboratório de Ecologia Aquática, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6681, 90619-900, Porto Alegre, RS, Brazil.

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The unicellular algae *Pseudokirchneriella subcapitata* (Korschikov) Hindák has been frequently used for ecotoxicological tests. In this paper, a calibration curve is proposed correlating absorbance values (684 nm) and cell density for routine ecotoxicological experiments. Density (cells/mL) could be estimated as follow: \( \text{Cell Density} = e^{\left(\ln(\text{absorbance}_{684}) + 16.439\right) / 1.0219} \) \((n=130; r^2=0.9998)\). Residual distribution revealed that the equation could be applied for algal densities under 5,000,000 cells/mL and/or absorbance values as high as 0.5.

Key words: *Pseudokirchneriella subcapitata*, spectrophotometry, ecotoxicological tests.

INTRODUCTION

Algae play a major ecological role in most aquatic ecosystems as dominant primary producers (Pfleeger et al., 1991; Lewis, 1995). Several species have been shown to be sensitive to toxics (Geis et al., 2000; Weyers et al., 2000), making this organisms widely recommended for ecotoxicological assays to evaluate toxicity of industrial wastewater or as bioindicators for chemical compounds present in water samples (Eaton et al., 1995). In this respect, the Chlorophycea *Pseudokirchneriella subcapitata* (Korschikov) Hindák (previously named *Raphidocelis subcapitata* and *Selenastrum capricornutum*) is one of the most frequently used algal species for toxicity tests (Nygaard et al., 1986).

Quantifying phytoplankton is usually done by time consuming methods, as direct cell counts under microscope or measurements of cellular mass or volume. Nevertheless, indirect methods that correlate algal density to light absorbance at specific wavelengths are not only reliable, but also easy to setup for automatic monitoring systems. So, the main goal of this work is to calibrate a regression model to estimate density of *P. subcapitata* in water samples by using spectrophotometry absorbance values.

MATERIALS AND METHODS

Ordinary data from routine chronic toxicity tests \((n=130)\) with *P. subcapitata* were used to calibrate a mathematical model to estimate the algal density as a function of light absorbance through spectrophotometry. Algal concentration was estimated by the mean number of cells obtained from direct cell count. Three subsamples of 1 mL each were screened using a counting chamber (Neubauer) and a light microscope (Zeiss Inc.) following McAteer and Davis (1994).

Maximum absorbance was inspected by scanning a culture sample between 600 and 800 nm (Cary 1E-Varian...
Figure 1. Pattern of light absorbance for a solution with P. subcapitata screened between 600 and 800 nm.

RESULTS AND DISCUSSION

Standard routines to estimate algal concentration include direct cell counts, chlorophyll content measurement, and absorbance or turbidity numerical correlations (EPA, 1994). When spectrophotometrical absorbance is the chosen method, a reading wavelength of 750 nm is usually recommended (EPA, 1994; Eaton et al., 1995), although values of 680 nm (Rojícková-Padrtová and Marsálek, 1999; Geis et al., 2000; Markle et al., 2000) and 687 nm (Valer and Glock, 1998) have also been used. These values are correlated to the light absorbance of chlorophyll, which could be best determined at a wavelength around 664 nm (Hersh and Crumpton, 1987; Fargasová, 1996; Rojícková-Padrtová et al., 1998). Figure 1 presents the pattern of light absorbance for a solution with P. subcapitata screened between 600 and 800 nm. Two peaks could be observed (624 and 684 nm), with the highest absorbance obtained at 684 nm, representing the wavelength of maximum sensitivity to quantify P. subcapitata samples. So on, all further analyzed samples were read in this wavelength.

Figure 2 shows the relationship between absorbance and cell density for P. subcapitata solutions. The gray line represents the adjusted absorbance equation:

\[
\text{Absorbance (684 nm)} = 7.2578 \times 10^{-8} \cdot (\text{Cells/mL})^{1.0219} \quad (r^2=0.9998).
\]

A power function \( y = a \cdot x^b \) was used instead of a simple linear coefficient as a way to minimize bias related to cell shading in increased densities. The identified \( b \) value, a little larger than one, indicates that absorbance does not increase linearly with cell density, but at increasing rates related to a cell to cell shading effect. Solving the former equation, the cell density (cells/mL) from absorbance values at 684 nm could be estimated as follow:

\[
\text{Cell Density} = e^{\left\{ \ln (\text{absorbance}_{684}) + 16.439 \right\}/1.0219}.
\]

Nevertheless, even with a high overall determination coefficient \( (r^2=0.9998) \), a biased absorbance response was identified when densities increased from 5 million cells/mL. Figure 3 shows the percentile deviation ([observed-expected]/observed.100) according to the adjusted model. Percentile deviations are under 2.5 (%) and with a random distributional pattern for cell densities.
Figure 2. Relationship between absorbance (684 nm) and cell density for *Pseudokirchneriella subcapitata* solutions. Black dots represent values obtained from routine toxicological tests (n=130) and the gray line represent the adjusted absorbance equation: Absorbance (684 nm) = 7.2578E^{-8}.(Cells/mL)^{1.0219} (r^2=0.9998).

Figure 3. Percentile deviation ([observed-expected]/observed.100) of the proposed model of absorbance (Absorbance (684 nm) = 7.2578E^{-8}.(Cells/mL)^{1.0219}) as a function of cell density of *Pseudokirchneriella subcapitata*.

up to 4.5 million (cells/mL). Between 4.5 and 5.0 million (cells/mL), percentile errors are all positive, but under the 2.5% threshold. Above 5.0 million (cells/mL) the adjusted model is strongly biased, with error increasing exponentially.

Valer and Glock (1998) had already presented equations to estimate algal concentrations from absorbance data for cell densities between $10^4$ and $10^5$ cells/mL. In
the present work, by using a power function, densities of P. subcapitata of up to 5,000,000 \((5 \times 10^6)\) cells/mL were precisely estimated. Nevertheless, the proposed equation is not recommended when the measured absorbance value exceed 0.5, which may require sample dilution.

REFERENCES


