Journal of Environmental Chemistry and Ecotoxicology Vol. 3(8), pp. 225-228, August 2011
Available online http://www.academicjournals.org/jece
ISSN-2141-226X ©2011 Academic Journals

Full Length Research Paper

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

Lúcia Helena Ribeiro Rodrigues[1*], Alexandre Arenzon[2], Maria Teresa Raya-Rodriguez[2] and Nelson Ferreira Fontoura[3]

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

Accepted 30 June, 2011

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: Cell Density = $e^{\{[\ln(\text{absorbance\_684}) + 16.439]/1.0219\}}$ ($n=130$; $r^2=0.9998$). 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 toxicants (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.

*Corresponding author. E-mail: luciarrodrigues@gmail.com.

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

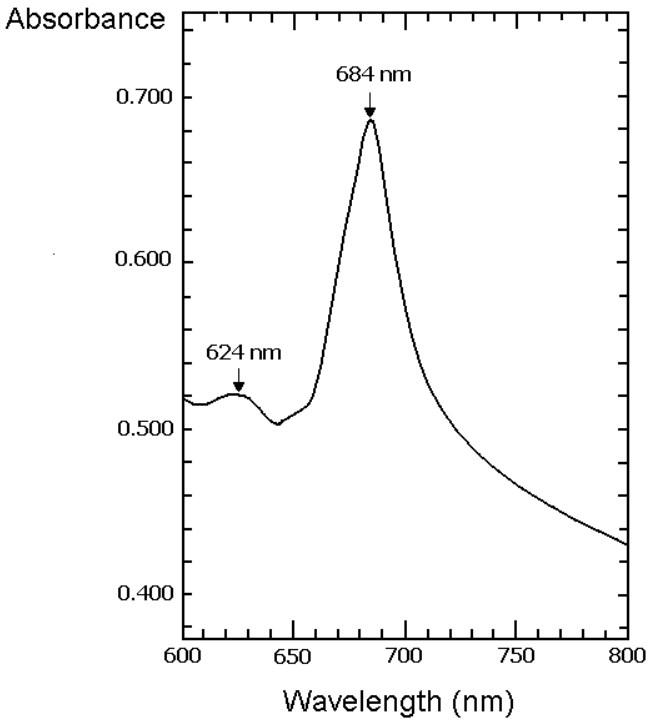

Figure 1. Pattern of light absorbance for a solution with *P. subcapitata* screened between 600 and 800 nm.

spectrophotometer). The highest absorbance value was then used to calibrate the curve of algal density. The relationship between spectrophotometer absorbance and the counted number of cells follows a general power equation:

$$\text{Absorbance} = a.(\text{Cells/mL})^{b}$$

where a and b are calibration coefficients, estimated using standard least squares procedures for linear regression after log transformation of absorbance and density data.

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 684nm, 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:

$$\text{Absorbance (684 nm)} = 7.2578E^{-8}.(\text{Cells/mL})^{1.0219} \quad (r^2=0.9998).$$

A power function ($y=a.x^{b}$) was used instead 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:

$$\text{Cell Density} = e^{\{[\ln(\text{absorbance\_684}) + 16.439]/1.0219\}}.$$

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

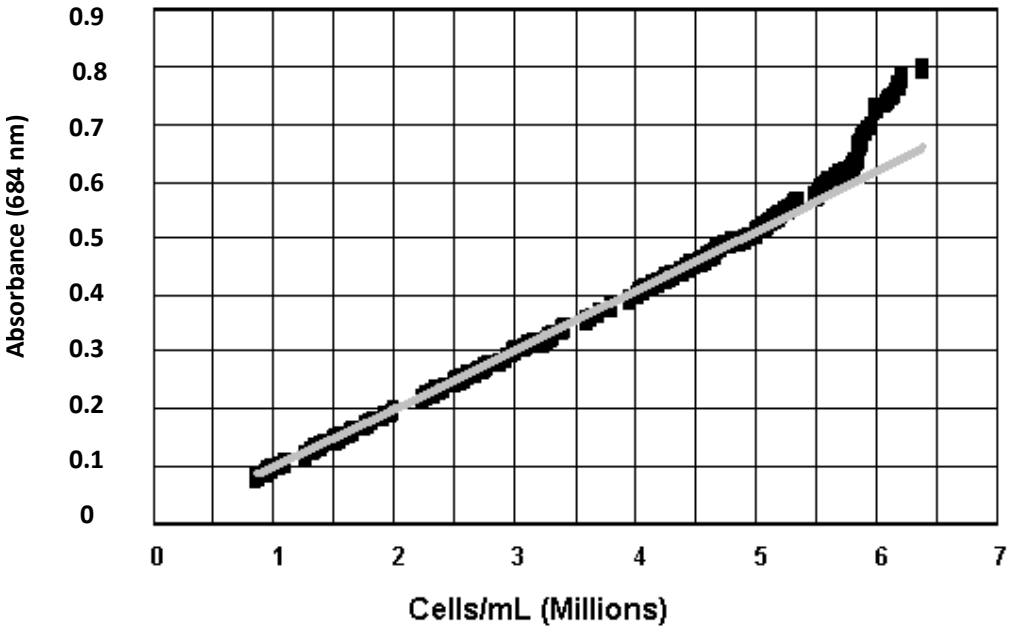

Figure 2. Relationship between absorbance (684 nm) and cell density for *Pseudokirchneriella subcapitata* solutions. Black dots represent values obtained from routine toxicological tests (n=130) and the gray line represent the adjusted absorbance equation: Absorbance (684 nm) = $7.2578E^{-8}.(Cells/mL)^{1.0219}$ (r^2=0.9998).

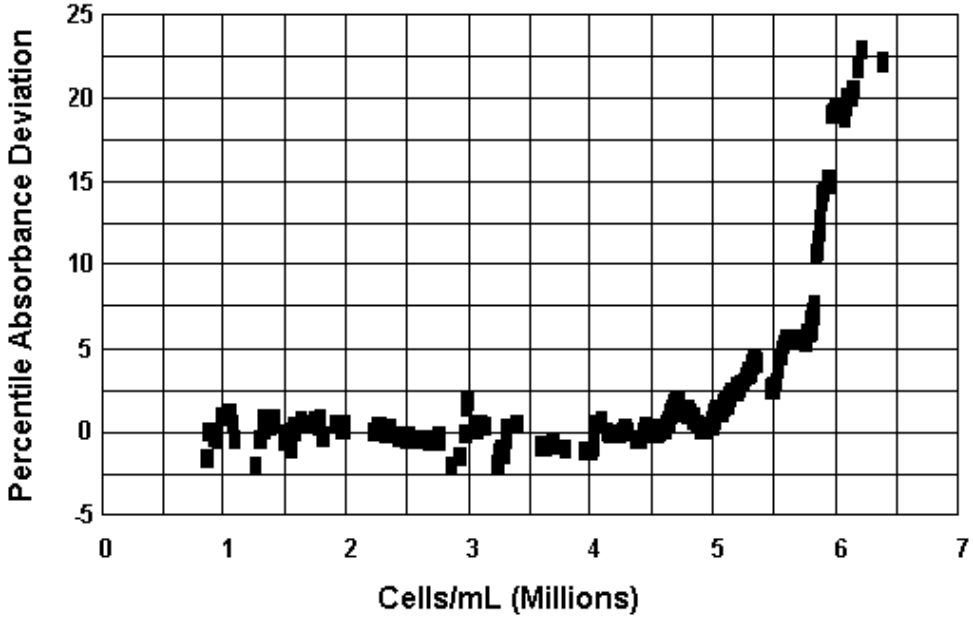

Figure 3. Percentile deviation ([observed-expected]/observed.100) of the proposed model of absorbance (Absorbance (684 nm) = $7.2578E^{-8}.(Cells/mL)^{1.0219}$) as a function of cell density of *Pseudokirchneriella subcapitata*.

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 ($5x10^6$) 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

Eaton AD, Clesceri LS, Greenberg AE (1995). Standard Methods for the Examination of Water and Wastewate .ed. American Plublic Health Association, Washington. p. 19

Environmental Protection Agency (EPA) (1994) United States. Short-term methods for measuring the chronic toxicity of effluents and receiving waters to freshwater organisms. 3ª ed. Cincinati, OH.: U.S. Environmental Protection Agency, 1994. EPA 600/4-91/002.

Fargasová A (1996). Inhibitive effect of organotin compounds on the chlorophyll content of the green fresh water alga *Scenedesmus quadriculata*. Environ. Contam. Toxicol., 57: 99 – 106.

Geis SW, Fleming KL, Korthals ET, Searle G, Reynolds L, Karner DA (2000). Modifications to the algal growth inhibition test for use as a regulatory assay. Environ. Toxicol. Chem., 19: 36-41.

Hersh CM, Crumpton WG (1987). Determination of growth rate depression of some of green algae by atrazine. Environ. Contam. Toxicol., 39: 1041 – 1048.

Lewis MA (1995). Use of freshwater plants for phytotoxicity testing: a review. Environ. Pollut., 87: 319–336.

Mcateer J, Davis J (1994). Basic cell culture technique and the maintenance of cell lines. In: Davis, J. Basic Cell Culture - A practical approach. Oxford: IRL Press. (The Practical Approach Series). pp. 109-143

Markle PJ, Gully JP, Baird PB, Nakada KM, Bottomley JP (2000). Effects of several variables on whole effluent toxicity test performance and interpretation. Environ. Toxicol. Chem., 19: 123-132.

Nygaard G, Komárek J, Kristiansen J, Skulberg OM (1986). Taxonomic designations of the biossay alga NIVA-CHL 1 (*Selenastrum capricornutum*) and some related strains. Opera Bot., 90: 1-46.

Pfleeger TG, Mcfarlane JC, Sherman P, Volk G (1991). A short-term bioassay for whole plant toxicity. In: Gorsuch, J.W., Lower, W.P., Wang, W., Lewis, M.A., (Eds.). Plants for toxicity assessment. STP 1115. American Society for Testing and Materials, Philadelphia, PA, 2: 355 – 364.

Rojícková-Padrtová P, Marsálek B (1999). Selection and sensitivity comparisons of algal species for toxicity testing. Chemosphere, 38: 3329 – 3338.

Rojícková-Padrtová P, Marsálek B, Holoubek I (1998). Evaluation of alternative and standard toxicity assays for screening of environmental samples: Selection of na optimal test battery. Chemosphere, 37: 495 - 507.

Valer RM, Glock L (1998). Quantificação de algas clorofíceas de interesse ecotoxicológico através do método espectrofotométrico. Acta Limnologica Brasiliensia, 11(2): 149 – 156.

Weyers A, Sokull-Kluttgen B, Baraibar-Fentanes J, Vollmer G (2000). Acute toxicity data: a comprehensive comparison of results of fish, Daphnia, and algae tests with new substances notified in the European Union. Environ. Toxicol. Chem., 19: 931–1933.