

Note

Cell shrinkage of *Isochrysis galbana* (Prymnesiophyceae) during storage with preservatives

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Abstract: Cell volume of *Isochrysis galbana* (Prymnesiophyceae) was determined with a microscope before and after fixation during storage at 4 and 25°C. The shrinkage effect at 4°C was not detectable but at 25°C. It was described by the exponential equation; $Y=0.797+0.224 \exp(-0.074 X)$ for 2% formaldehyde and $Y=0.849+0.150 \exp(-0.87 X)$ for 1.6% Lugol's solution where X is hours and Y is the ratio of fixed volume to live volume. Lugol's solution provides 6.5% less shrinkage than formaldehyde ($p<0.05$). We recommend Lugol's solution as a preservative at 25°C with a shrinkage correction or refrigeration for storage at 4°C, otherwise a species-specific correction should be made for estimating live volume from fixed material.

Key words: cell volume, formaldehyde, Lugol's solution, storage temperature

Estimation of cell volume is a fundamental approach to understanding marine microbiology and ecology. Once cell volume is obtained, it can be converted to carbon biomass. Production dynamics of marine ecosystems can not be fully understood without information on the amount and distribution of biomass. Automated determination of cell volume, such as by using electronic particle counters (Sheldon & Parsons 1967) and image analysis (Sieracki et al. 1989), usually provides on accurate estimation of cell volume although it can be expensive and may provide inaccurate or biased volumes for non-spherical cells (Grover et al. 1982). As microscopy is relatively inexpensive and technically simple, the microscope is and will likely continue to be one of the most common tools for phytoplankton biomass evaluation. For field samples, microscopic determination has the advantage over automated determination that it provides information on the species composition and cell volume of each species. For culture samples, volume estimation is usually done on cells that have been preserved by fixatives except for the case of well equipped laboratories with automatic cell counters. An obvious effect of preservatives on reducing the cell volume has been recognized for phytoplankton (Booth 1987, Montagnes et al. 1994). A general correction factor has been provided for estimating live volume from fixed material after 72 h of preservation (Montagnes et al. 1994) although they recognized that cell shrinkage occurred dramatically within the first 24 h and at a much reduced rate thereafter. Correction factors including this initial

effect have not been considered in previous studies. We have investigated the possible effects associated with preservatives and storage temperature, including frequent observations during the period of initial shrinkage, to establish an accurate correction factor for *Isochrysis galbana* Parke (Prymnesiophyceae).

Isochrysis galbana is widely employed as a model organism for various studies on biomass production and physiological characterization (e.g. Sanchez et al. 2000). This species was chosen as an experimental organism because of its spherical shape, which reduces any possible inaccurate estimation of cell volume due to easy and accurate approximation using microscopy (Hillebrand et al. 1999). Cells were grown in filtered seawater (Salinity 35) enriched by f/2 medium (Guillard & Ryther 1965) in batch mode in 4L bottles at 25°C under a 12 h light and 12 h dark cycle. Light was provided by fluorescent tubes (Toshiba FL40SW, Tokyo, Japan) at $200 \mu \text{ photons m}^{-2} \text{ s}^{-1}$, as determined by a Biospherical Instrument 4π irradiance meter (QSL-100) in a temperature–light controlled incubator. Cells were harvested at the middle of the logarithmic growth phase. As Hillebrand et al. (1999) recommended, linear dimensions of cells that had been cooled by refrigeration to 4°C to slow cell mobility were measured before the experiments as an estimation for live cells. Harvested cells were preserved with 2% formaldehyde (Olivieri 1985) or 1.6% Lugol's solution (Thronsen 1978). Preserved cells were divided into two fractions; the first fraction was kept at 4°C and the second fraction was kept at 25°C in dark conditions. Subsamples were taken every 4 h for the first 24 h (6 occasions), and every 24 h for the

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next 648 h (28 occasions). More than 50 live or fixed cells were measured for each subsample with an ocular micrometer using an Olympus inverted microscope (IMT-2)(Lund et al. 1958). Linear measurements were made from the edges of the thin outer cell layer. Volume estimates were made from linear dimensions using the appropriate geometric shapes (Hillebrand et al. 1999). Cells shrank substantially over the first 24 h and shrank at a much reduced rate thereafter in both formaldehyde (Fig. 1) and Lugol's solution (Fig. 2) when the samples were stored at 25°C. From 48 to 672 h, cells shrank at a nearly constant rate. The time-dependent shrinkage can be expressed with the following exponential equations. For formaldehyde,

$$Y = 0.797 + 0.224 \exp(-0.074 X) \quad (1)$$

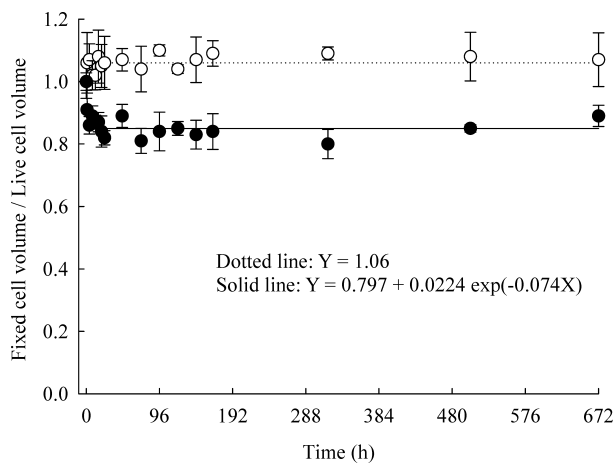


Fig. 1. Temporal change in the ratio of fixed volume to live volume based on fixation with 2% formaldehyde at 4°C (open circles with dotted line for the mean) and 25°C (solid circles with solid line for the equation (1)).

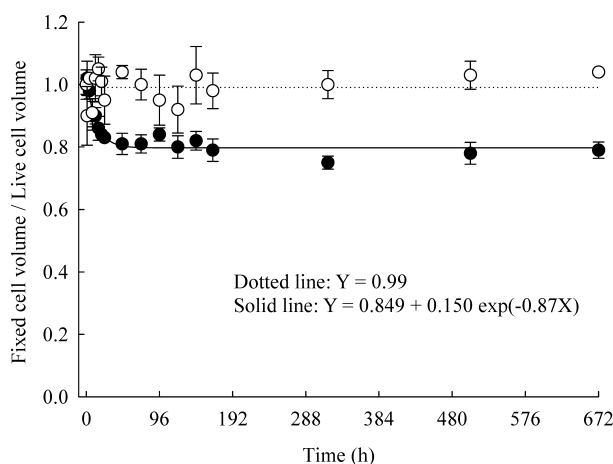


Fig. 2. Temporal change in the ratio of fixed volume to live volume based on fixation with 1.6% Lugol's solution at 4°C (open circles with dotted line for the mean) and 25°C (solid circles with solid line for the equation (2)).

and for Lugol's solution,

$$Y = 0.849 + 0.150 \exp(-0.87 X) \quad (2)$$

where X is hours and Y is the ratio of fixed volume to live volume. Both fits are statistically significant at the level of $p=0.001$. Differences in the effect of preservatives were observed. At 25°C, Lugol's solution gives a gentle slope during the first 48 h and 6.5% less shrinkage than formaldehyde during the period from 48 to 672 h ($p<0.05$). Cells did not shrink significantly and cell volume was virtually unchanged when samples preserved in either formaldehyde (mean+one standard deviation= 1.06 ± 0.03) (Fig. 1) or Lugol's solution (0.99 ± 0.03) (Fig. 2) were kept at 4°C.

We recommend Lugol's solution as a preservative because it leads to significantly less shrinkage than formaldehyde at 25°C or during storage at 4°C. Otherwise a species-specific correction should be applied for the estimation of live volume from fixed material because a species-specific effect of preservatives has been reported in a study when four representative species were compared (Montagnes et al. 1994).

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