Leveling the gap between different counting techniques in coccolithophore cultures

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Monospecific cultures of coccolithophores are fundamental to exploring species-specific response to various controlling parameters (temperature, pH, CO_2, etc.). Acquiring the most accurate values for cell counting is fundamental to correctly quantifying the maximum cellular density and growth rate, which are pivotal for studying the culture response, especially when it comes to comparing the results among different laboratories. We tested two of the most commonly used cell counting methods to identify which approach has the highest accuracy and speed for evaluating a culture's cellular density (cells/mL): Sedgewick Rafter chamber or Bürker counting chamber. Moreover, because manual cell counts are time consuming, we compared them with the culture absorbance (i.e., optical density [OD]) measured with a spectrophotometer, a poorly used method for coccolithophores but extremely useful for high density cultures and to speed up the counting process. These methodologies were tested on three different species of coccolithophores: *Helicosphaera carteri*, *Chrysotila carterae*, and *Emiliania huxleyi* (from Roscoff Culture Collection) that cover a wide range in cellular densities and cell sizes, from lower cell density (~3×10^5 cells/mL) and larger size (20 µm) for *H. carteri* to higher cellular density (>5×10^6 cells/mL) and smaller size (5 µm) for *E. huxleyi*.

The preliminary data document that the Sedgewick Rafter chamber can be confidently used from very low cellular densities (e.g., 500 cells/mL) to higher counts (~3×10^5 cells/mL), maintaining a low standard deviation among the replicates. Above this upper limit, culture dilution is necessary when using the Sedgewick Rafter chamber to avoid increasing errors from the human operator. Dilution at higher cellular densities can be avoided using the Bürker chamber, which also returns good results with low standard deviations for cellular densities above 10^6 cells/mL and for small-sized species. Indeed, small-sized species, such as *E. huxleyi*, can make the manual counting more difficult with the Sedgewick Rafter chamber, which has fewer guide grids compared to the Bürker chamber. All the above-mentioned counts can be linearly correlated with OD measurements at a specific wavelength (750 nm) according to the Lambert-Beer law within the validity range of 0.1–1. Both manual counting methods provided good coefficients of determination (>0.9 when correlated with the OD), indicating that the number of cells is well described by OD in the selected range.

Our data show that the Sedgewick Rafter chamber is an effective method for physiological studies, where lower cellular densities are commonly used. On the other hand, the Bürker chamber is more suitable for higher cellular densities required for biotechnological studies. Finally, although the OD is not commonly used for monitoring coccolithophore growth, we recommend the use of OD measurements to speed up data collection, particularly for high cell density studies that focus on coccolithophore productivity for practical applications.

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