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Cell homogenization and subcellular fractionation in two phytoplanktonic algae: implications for the assessment of metal subcellular distributions

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Appendix

Chemical composition of culture media is described in Table A1.

The cell disruption efficiency of a vortex-agitator with beads, the Disruptor Genie beadbeater (Scientific Industries, model SI-D236), was first tested and optimized with respect to different parameters for *C. reinhardtii* and *P. subcapitata*. Zirconia-silica and glass beads of different diameters (0.1 and 0.5 mm) were tested for different agitation times (1 to 15 min). Beads/solution ratios (vol/vol) were also varied from 50% to 95% as described in Table A2. The Eppendorf tubes were kept on ice before homogenization and after each minute of disruption.

Sonication efficiencies for *P. subcapitata* calculated with the ^{14}C uptake or the particle counter method were not significantly different for all the ^{14}C exposure times tested (Table A3) ($P > 0.05$). The exposure time could have affected the homogenization efficiency evaluation by the ^{14}C technique due to the incorporation of ^{14}C into membranes and cell wall debris (>2 µm) retained on filters after disruption which would contribute to the ^{14}C intact cells signal. If this were the case, for shorter exposures the proportion of ^{14}C incorporated into the fraction retained by the filters should decrease and the homogenization efficiency should artificially increase. This was not observed, and the *P. subcapitata* algae cells remaining after sonication thus clearly seem to be intact.

As seen in Table 1, *C. reinhardtii* cells are much easier to break than *P. subcapitata* cells. This observation is likely linked to the cell wall composition of the two species. The cell wall of *C. reinhardtii* does not contain cellulose or other polysaccharides, in contrast to the *P. subcapitata* cell wall (Hoek et al. 1995). The cell wall of *C. reinhardtii* is instead composed of several layers of hydroxyproline-rich glycoproteins (Voigt 1988).

Sonication was also tested by Eixler et al. (2005) to extract total polyphosphate granules and total cellular phosphorus from *Chlorella vulgaris*, a green alga with cellulose-rich cell walls. A sonicator equipped with a probe was used at full power for 5 min. Epifluorescence microscopic analysis of DAPI-stained algae showed a high proportion of intact cells with yellow fluorescing granules (at 526 nm under UV excitation) remaining inside the cells. Only 33% of the total cellular phosphorus could be measured after the sonication. Similar breakage efficiencies were obtained in our laboratory with the species *Chlorella fusca var. vacuolata* (data not shown). Thus, there is some precedent with other green algae with cellulose-rich cell walls for the low sonication efficiency that we obtained for *P. subcapitata*.

Several authors have reported high breakage efficiencies for diatom cells using sonication (>95%), albeit under more harsh conditions than those used in this study (Connell et al. 1991, Martinson et al. 1998, Holm et al. 2008). However, such treatments could affect organelle integrity, as was pointed out by Martinson et al. (1998). These latter authors showed that electron transport activity in thylakoids of the diatom *Cylindrotheca fusiformis* could be reduced by around 80% after harsh sonication (six to eight cycles of 15-s bursts at 105 W and a centrifugation between each cycle) to achieve complete cell breakage.

Table A1. Total molar concentrations of culture medium constituents (MHSM-1) and rinse solution (MHSM-R) derived from the HSM medium of Macfie et al. (1994).

Ions	HSM	MHSM-1	MHSM-R
NH_4^+	9.35×10^{-3}	9.37×10^{-4}	9.37×10^{-4}
Cl^-	9.49×10^{-3}	5.98×10^{-6}	—
K^+	2.20×10^{-2}	4.22×10^{-3}	4.22×10^{-3}
PO_4^{3-}	1.37×10^{-2}	1.37×10^{-4}	1.37×10^{-4}
CO_3^{2-}	Atm	Atm	Atm
NO_3^-	—	5.07×10^{-3}	5.07×10^{-3}
SO_4^{2-}	8.12×10^{-5}	8.12×10^{-5}	8.12×10^{-5}
Mg^{2+}	8.12×10^{-5}	8.12×10^{-5}	8.12×10^{-5}
Ca^{2+}	6.80×10^{-5}	6.80×10^{-5}	6.80×10^{-5}
Na^+	1.02×10^{-4}	1.02×10^{-4}	1.02×10^{-4}
BO_3^{3-}	3.01×10^{-6}	3.01×10^{-6}	
Mn^{2+}	2.10×10^{-6}	2.10×10^{-6}	
EDTA	8.06×10^{-7}	8.06×10^{-7}	
Fe^{3+}	5.92×10^{-7}	5.92×10^{-7}	
MoO_4^{2-}	3.00×10^{-8}	3.00×10^{-8}	
Zn^{2+}	2.43×10^{-8}	2.33×10^{-7}	
Co^{2+}	1.09×10^{-8}	1.09×10^{-8}	
Cu^{2+}	7.04×10^{-11}	$>2.52 \times 10^{-10}$	
		$<1.04 \times 10^{-9}$	
HEPES		10×10^{-3}	

The carbonate concentration is presumed to be at equilibrium with the atmosphere. The pH is maintained at 7.0 with HEPES (3-[4-(2-hydroxyethyl)-1-piperazinyl] propanesulfonic acid) and the free metal concentrations are buffered with EDTA (ethylenediaminetetraacetic acid). Targeted total metal concentrations (Mn, Zn, Fe, Co) in MHSM-1 culture medium (calculated from the trace metal AAP stock solution) were effectively measured by ICP-MS or ICP-AES. Analytical results indicated that impurities from salts did not contribute to these four metal concentrations in MHSM-1 culture medium. The Cu^{2+} concentration in MHSM-1 medium was below the ICP-MS detection limit (1.04×10^{-9} M), but the nominal concentration was 2.52×10^{-10} M (concentration calculated from the AAP trace metal stock solution). This uncertainty regarding the Cu concentration did not affect the free Cd^{2+} concentrations as calculated with MINEQL+.

Table A2. Weight of glass and zirconia-silica beads (diameter 0.5 mm) introduced into each 1.5-mL Eppendorf tube to reach different beads/solution volume ratios.

Beads/solution ratio, % vol/vol	Volume of culture medium, mL	Volume of beads, mL	Weight of glass beads, g	Weight of zirconia-silica beads, g
50	0.600	0.300	0.750	1.110
60	0.600	0.360	0.900	1.332
70	0.600	0.420	1.050	1.554
80	0.600	0.480	1.200	1.776
90	0.600	0.540	1.350	1.998
95	0.600	0.570	1.425	2.109

The specific gravity of glass and zirconia-silica beads is 2.5 and 3.7 g cm^{-3}, respectively.

Table A3. Two different methods to estimate cell disruption efficiency by sonication as percentages of broken cells for *P. subcapitata* after different ^{14}C exposure times (n = 3, mean ± standard deviation).

Exposure time, min	Disruption efficiency, %	
	^{14}C	Particle counter
7	32.6 ± 6.8	19.2 ± 3.1
25	18.6 ± 11	20.1 ± 4.5
49	25.5 ± 4.3	22.3 ± 6.3
68	33.5 ± 13.3	22.3 ± 3.4

Sonication conditions: 4 min, 22 W, 0.2 s s^{-1} pulsation frequency.

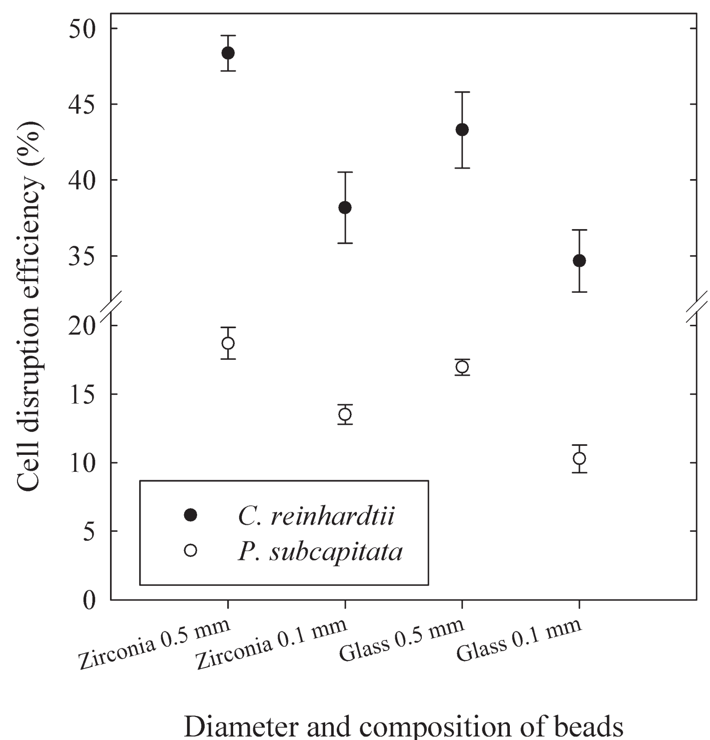

Fig. A1. Cell disruption efficiency (% of disrupted cells calculated with a Coulter Counter) for *C. reinhardtii* and *P. subcapitata* obtained with a beadbeater using beads of different diameter (0.5 and 0.1 mm) and composition (zirconia and glass). *C. reinhardtii* and *P. subcapitata* cells were disrupted during 1 and 6 min, respectively, with a beads/solution ratio of 90% vol/vol. Error bars are the standard deviations around the mean of three replicates.

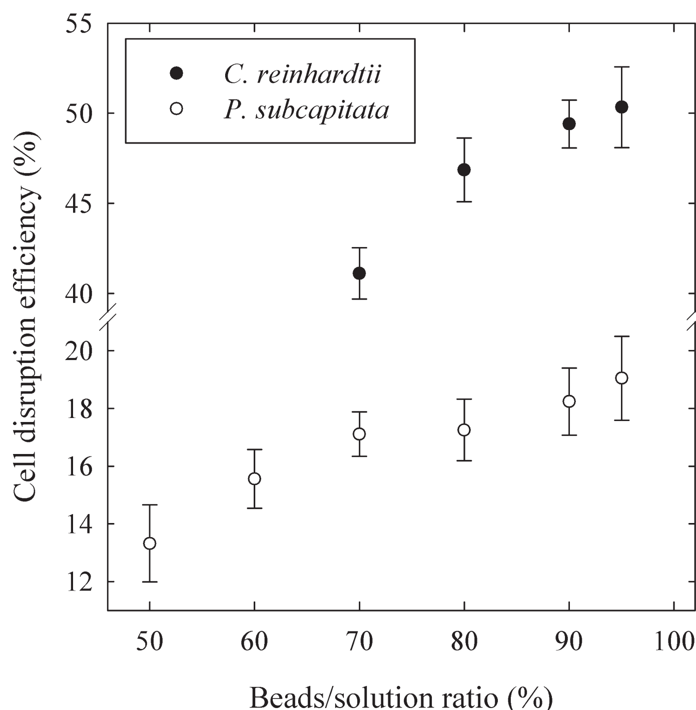

Fig. A2. Cell disruption efficiency (% of disrupted cells calculated with a Coulter Counter) for *C. reinhardtii* and *P. subcapitata* obtained with a beadbeater using different beads/solution volume ratio varying from 50% to 95% vol/vol for *P. subcapitata* and from 70% to 95% vol/vol for *C. reinhardtii. C. reinhardtii* and *P. subcapitata* cells were disrupted during 1 and 6 min, respectively, with zirconia beads of 0.5 mm. Error bars are the standard deviations around the mean of three replicates.

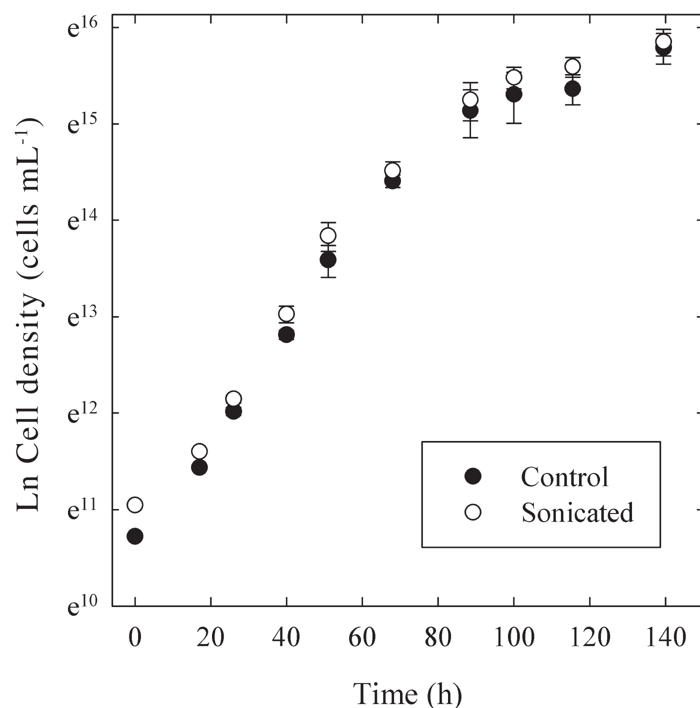

Fig. A3. Natural logarithm of cell density (cells mL^{-1}) as a function of time (h) for *P. subcapitata* grown in MHSM-1 culture medium. Control and sonicated cells (4 min, 22 W, 0.2 s s^{-1} pulsation frequency) in MHSM-1 culture medium were inoculated at a cell density of around 40,000 intact cells mL^{-1} (time 0). Density of intact cells in sonicated samples was calculated by using the disruption efficiency calculated with the Coulter Counter technique (Table 1; Table A3) to determine the volume required to inoculate each fresh culture medium. Error bars are standards deviations around the mean of three replicates.

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