Changes to the elemental composition of the green alga *Chlamydomonas reinhardtii* based on variations in cultivation conditions

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Project Goals: The LLNL Biofuels SFA is developing advanced methods to support sustainable microalgae fuel production. In this part of the project we are working on characterizing metal metabolism in microalgae through imaging. Metals are cofactors for essential enzymes and the ability to localize and quantify them will lead to a better understanding of how microalgae metal metabolism works. Because metals occur at parts per million and lower levels in some cells, they can be difficult to image by standard methods. Here we use high spatial resolution secondary ion mass spectrometry with a Cameca NanoSIMS 50 to image metals in embedded and sectioned cells. The NanoSIMS data are correlated with other methods to provide a more complete understanding of metal metabolism in these microalgae.

Trace metals are critical for many essential reactions in all living organisms. Among the most important metals are copper (Cu), iron (Fe), and manganese (Mn), which all serve as cofactors to enable catalysis and redox chemistry. For decades, the unicellular green alga *Chlamydomonas reinhardtii* has been used as a reference organism for understanding eukaryotic metal homeostasis because it retains many genes that were present in the common ancestor to both plants and animals, and it is amenable to classical and molecular genetic manipulation. The elemental composition in Chlamydomonas cells can be altered by nutrient availabilities in the environment (e.g. trace metal abundances, carbon/nitrogen supply). In addition, growth parameters such as cell density, carbon/energy source, soil/water pH and aeration are known to influence the metabolism of the alga, which might affect the trace metal quota. To understand the potential impact of such growth parameters on metal homeostasis in Chlamydomonas, we have completed a systematic analysis of cellular Cu, Fe, and Mn content as a function of six different cultivation variables using ICP-MS/MS. These variables include 1) cell density/sampling time, 2) starting medium pH, 3) photon flux density, 4) vessel size, 5) culture volume, and 6) shaker

speed. Chlamydomonas cells accumulate Cu and Fe, but not Mn, as a function of time, especially during the stationary phase. Alkaline medium (pH 8.5) under photoheterotrophic growth condition induced hyperaccumulation of Fe in cells up to 10- to 20- fold higher than cells grown in the neutral pH medium. Other parameters like aeration and photon flux density have an impact on Cu, Fe, and Mn quota, but these effects are smaller compared to the influence of pH on Fe accumulation. Our results suggest that the regulation of individual trace metals (Cu, Fe, and Mn) in Chlamydomonas may each be specific in response to different environmental stimuli. Furthermore, this analysis demonstrates that cell density/timing of sampling and medium pH must be firmly controlled during experimentation in order to ensure a consistent elemental makeup of the cells, and thereby yielding reproducible and reliable outcomes. Meanwhile, because the accumulation of Fe in alkaline condition was so striking, we further investigated this aspect in more detail. To examine the effects of medium pH on cell physiology, we have undertaken a comparative transcriptome analysis of cells grown in media at pH 8.5 versus pH 7.0. We observed that 2523 genes (~14%) are differentially regulated between cultures grown at alkaline vs. neutral pH. We also visualized Fe distribution in cells sampled at alkaline and neutral pH by nanoSIMS. Fe was evenly distributed within the cell when grown in neutral pH media. In comparison, imaging of cell sections sampled at pH 8.5 showed distinctive hotspots of Fe colocalized with calcium and phosphorus. This suggests that the excess Fe was stored in acidocalcisomes, a lysosome-related organelle.

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