

Novel Iron Storage Forms and Mn, Cu and Zn fingerprints in Algae of Potential Biotechnological Importance

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Algae are of cardinal importance for oceanic ecology and geochemistry [1-3]. Brown algae (phaeophyta) are gaining an increasing significance as a resource of organic matter for industrial applications (alginate, fucanes) and for the production of biofuels. Multicellular macroalgae (seaweeds) have various advantages over classical crop plants for the production of biofuels. The high biomass yields do neither require arable land nor additional H₂O. Algae can be engineered to increase photosynthetic efficiency. In addition, a sufficient supply of nutrients will also increase the biomass yield.

In particular, the iron level in ocean waters is even lower than in most terrestrial habitats due to both the low solubility of Fe(III) in oxic seawater and the fact that a large fraction of the limited iron available is already tightly complexed (5). Thus, iron availability is now known to limit primary productivity in certain oceanic regimes. However, unspecific fertilization techniques employing ferrous sulphate exhibit serious drawbacks such as eutrophication, and toxin production caused by the growth of undesirable species. For specific iron fertilization, a precise knowledge of iron uptake mechanisms and of substrate dependent iron metabolism is essential.

One major project objectives was, therefore, the determination of the iron acquisition and storage strategy of the macroalga *E. siliculosus*. For these studies, Transmission Mössbauer Spectroscopy (TMS) and EXAFS investigated frozen whole cell samples, alongside with biochemical approaches [4].

Analysis of TMS identified two different iron species: an iron-sulphur (Fe₄S₄) and a polymeric FeO₆ species. These models were used to simulate the EXAFS spectra by FEFF and the Athena/Artemis package [5]. The two component fit exhibits a Fe-oxo species containing 3.5 ± 0.5 P and 1 ± 0.5 Fe atoms in the second coordination sphere, consistent with TMS and a polymeric P containing Fe-oxo species as typically found in biological Fe storage systems.

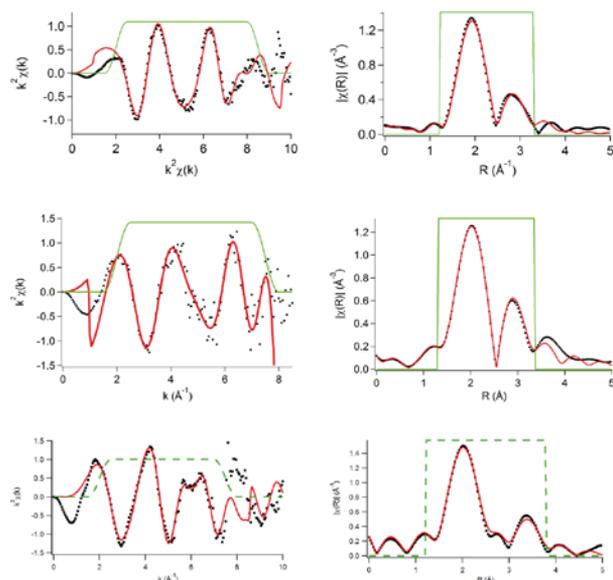
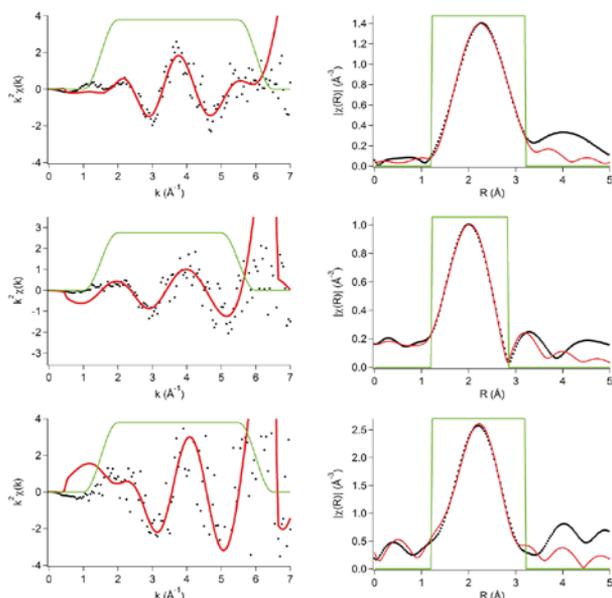


Figure 1: EXAFS Fe data of *Ectocarpus siliculosus* (top), *Emiliania huxleyi* (middle) and *Tetraselmis suecica* (bottom). The left column gives the experimental data (black dots) and the model fit (red line) in a Hanning type window (green line). The right column shows the experimental data (black dots) in R-space with the modelled fit (red line) and the fitting window (green line).

In addition, the relevance of other micronutrients - in particular of transition metals - in algae is merely partially understood. Therefore, a second goal of this project was the elucidation of transport, metabolization and functions of various transition metals (Mn, Cu, Zn) in a variety of algal species (*Ectocarpus siliculosus*, *Emiliania huxleyi*, *Tetraselmis suecica*).

For this part, frozen whole cells were investigated by EXAFS. In the case of Fe and Zn EXAFS reasonable data was collected and analysed (see Fig. 1 and 2 and [4, 6, 7]). In the case of Mn signals with a low signal to noise ratio could be detected. For Cu no signal was observable.

Alongside with TMS the Fe spectra reveal different iron storage mechanism for these three algae. In *E. siliculosus* the majority component consists out of polymeric Fe(III)-oxo species. In the case of *E. huxleyi* two different Fe-oxo species were found. One consists out of a Fe-O-P species and the other out of a Fe-O-Fe species, consistent with observed hyperfine splitting distributions in TMS. For *T. suecica* a $\text{Fe}_4\text{S}_4^{2+}$ cluster and a Fe-oxo species with a low P content were identified.



The EXAFS spectra of the three algae show different distances for the first coordination sphere. Therefore different model systems were used to fit the data. Each model system was extracted from known protein structures (Protein data Bank) and used as fit model without changing the number of ligands or intramolecular distances. The Zn EXAFS spectra of *E. siliculosus* was fitted with the model of a metallothionein and give a considerable well agreement with the data. In the case of *E. huxleyi* a Zn cluster as it is found in 1HZY was used as fit. In *T. suecica* a Zn-S cluster is consistent with the data.

Figure 2: EXAFS Zn data of *Ectocarpus siliculosus* (top), *Emiliania huxleyi* (middle) and *Tetraselmis suecica* (bottom). The left column gives the experimental data (black dots) and the model fit (red line) in a Hanning type window (green line). The right column shows the experimental data (black dots) in R-space with the modelled fit (red line) and the fitting window (green line).

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