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Biogeosciences Discussions

Interactive comment on "Impact of nutrient starvation on the biochemical composition of the marine diatom *Thalassiosira weissflogii*: from the whole cell to the frustule fraction" *by* C. Soler et al.

Anonymous Referee #1

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The aim of this paper is to link the exclusion of required nutrients to the silica and organic matter composition of a diatom and its frustule. This is an interesting paper that falls within the scope of this journal. I can appreciate the substantial amount of work that the authors have done for this paper. This project is a good example of how infrared spectroscopy can be used to measure changes in algal biochemistry. Particularly good was the implementation of algal PAM fluorescence to determine when FTIR measurements should be taken. Adding physiological measurements to algal cellular changes greatly helps in interpreting nutrient starvation impacts. However,

there is a large issue in this paper concerning FTIR data analysis, and one potentially major problem with data collection that may substantially affect all FTIR results.

Mixing ground frustules with KBr in known quantities is a valid way to try to standardize sample thickness across samples, but it appears too much sample relative to KBr may have been used. The largest issue in this paper is noted in the spectral absorbance values in figures 3 and 5, with absorbance as high as 5 in figure 3, and 3 in figure 5. Optimum FTIR absorbance values are from 0.4-0.75, but absorbance up to 1.25 may be reliably used.. Thus, if these are the true absorbance values, then the assumption of linearity in peaks over $\sim$2 is highly unlikely and quantitative measurements such as peak ratios cannot be made from these spectra. Absorbance is a logarithmic scale and at an absorbance of 1, only 10% of the light is reaching the detector. Thus an absorbance of 3 would mean only .1% of the light reaches the detector greatly reducing the signal to noise ratio. If this is the case, then the relative amount of silica in these samples would likely be substantially underestimated relative to other components like lipids or proteins. This non-linearity seems evident in Figures 7 and 8. It does not seem likely that after sonication and acid exposure, the organic matter content of frustules would be as high as 65% (p. 5969, Line 16). Unfortunately if non-linear absorbance is the case, the FTIR calculations for much of the results are not correct. If this is not the case, the authors must show data that linearity at such high absorbance are maintained in these samples.

A second issue is the analyses of the spectra. It is not correct to add up the area under the peaks and use the value as 100% of the cell contents to calculate 'global compositions' (p. 5964, Line 1). Not every molecule in the cell has a vibrational frequency in the mid-IR spectrum. And a single macromolecule can contribute to absorbance in various parts of the spectrum. For example the amide I (e.g. from C=O, C=C) and amide II (N-H, C-N) peaks contain different vibrations from the same proteins. Typically only one of these amide peaks is used to distinguish relative protein concentration. E.g., commonly, when water creates noise in the amide I region, the amide II peak only is

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used to assess protein. Therefore the FTIR results section and figures 4, 7, and 8 are likely not accurate and should be reanalyzed using a single peak for each major macromolecule. The change in ratios within a spectrum can be accurately assessed as FTIR gives us just the relative amount of each macromolecule. Creating a consistent thickness by mixing known amounts with KBR will enable direct comparison of peak absorbance values among samples. Perhaps comparing the spectra with principal component analysis to detect differences among treatments would work here.

Additional spectral identification issues are in the 1470 - 1300 cm-1 region, which is referred to here as the 'amines'. This region is quite complicated and also has absorbance from methyl and methylene groups of lipids, among other possible vibrations. Related to this, the summing of peaks 3 and 4 to get a protein measurement in figure 5c is not appropriate. This new peak is likely a different molecular vibration. Both peaks 3 and 4 appear to be evident as shoulders on the new at ∼1440 cm-1. It appears that a phosphodiester peak (P=O) may be present around 1220cm-1 (figure 3). This peak should be addressed as P limitation was one of the treatments.

This is an interesting topic and good application topic of FTIR. However the FTIR methodological problems and spectral analysis would have to be addressed before publication can be recommended.

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