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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 #2

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The authors of the present manuscript tried to analyze different frustle fractions of a diatom with respect to their macromolecular composition. From a scientific view, this is very interesting for the understanding of diatom physiology since diatoms play a major role in the global carbon cycle, not only for the capture of c02 but also as long term CO2-sink within the ocean. The frustules are one of the key players for the transport of organic C into deep zones of the water body. The use of FTIR-spectroscopy is logic, because there are no other methods available for the determination of macromolecular pools of very small volumes. The results show one the first view, that large parts of

the frustules are organic compounds. The ratios of these compounds are not surprisingly dependent on the nutrient availability. The Authors demonstrated these nutrient dependent changes and discussed them with respect to several known physiological features like e.g. carbon excretion and a stopped cell cycle. However, there are some major critical points that have to be addressed:

The FTIR-Spectra are of reasonable quality but however, the spectra interpretation needs a lot more of caution. Macromolecule quantification has been performed by peak integrals and computed on a percentage scale. This is unfortunately an oversimplification since it is assumed that all macromolecules have the same absorption properties (absorption coefficient). In fact this is not the case. The comparison of two reference substances may show that with the equivalent amount of sugar and protein, the peak maxima of the sugar spectra will be aprox 3 times larger than the protein peaks. Moreover if a concentration dependent calibration of reference substances will be measured, it is obvious not possible to reach the same slope of the calibration curve. On the basis of these essential physical properties, a quantification of macromolecular ratios with peak integrals is just a rough approximation and should be reanalyzed.

The second major critic point is the separation of carbohydrate and silica from the whole spectra. It is shown in figure 5b to what extent SiO2 account for the absorption within the 1300-1000 cm-1 the same region that has been used for the quantification of carbohydrates. The same holds true for the amid I band which is overlapping with vibrational bands of carbohydrate. The Integral of one absorption band is always a mixture of more than one macromolecule. Therefore, the quantification biased on peak integrals of protein, carbohydrate and silica is again an approximation and should be named as ratios of peak integrals rather than protein:carbohydrate ratio.

Third point: Some of the data needed to be explained in detail. I) The vibrational band called 3+4 in figure 5c has been annotated as proteins. This seems more speculation than a hard fact, since protein bands are very well characterized, whereas the region between 1500-1300 cm-1 is rather complex and not well characterized. Following this...

BGD

7, C2465–C2469, 2010

II) The ratio of protein:Silica in figure 7 for the FBW fraction showed more than 80% protein to around 10-15% Silica. This is a very hard contrast to the whole cell ratios of about ∼40% protein to 12% silica. Either there is only half of the silica chemically bound to the frustle and all of the cell protein (even the physiological active proteins) is fixed to the frustle, or there must be a chemical miracle that explains the increase in proteins. Here it is obvious, that point I) should be analyzed in more detail!

In conclusion, there are some points that have to be worked on. Unfortunately it is not easy to validate the macromolecule data by conventional chemical analysis, what may help to interpret the FTIR-Spectra. However, the use of reference spectra with a concentration depending calibration should help to determine real macromolecule ratios. The existing spectra however, can be used but at this stage the macromolecule contents should not named as quantified but titled as band-ratios.

There are some minor comments

1. M&M Since the macromolecule composition is dependent on the light phase, the time point of sampling should be stated.

2. Page 5964:5 The annotation of amine vibration should be cited.

3. Page 5970:5-30 The order of the results should be changed to fit into figure 7

4. Page 5975:2ff The excretion of CH is an assumption. Since the e-transport is reduced, it is more likely an effect of just less fixed carbon, whereas the already fixed carbon is bound into proteins. The same fact is again on Page 5976:20-25

5. Page 5977:1-3 Some data on cell numbers could help to understand this phenomenon

6. Page 5978:12-13 figure 7 shows more protein than carbohydrates in contrast to the stated carbohydrate as most dominant component

7. Page 5978:24-25 It is stated that the contribution of Si is reduced which is very

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7, C2465–C2469, 2010

unlikely. Instead the changed ratios arise from an increased protein content.

8. Page 5978:27-28 The excretion of carbon is just an assumption.

9. Figure 3: The sampling time for each spectra should be stated

1. Does the paper address relevant scientific questions within the scope of BG?

-> yes

2. Does the paper present novel concepts, ideas, tools, or data?

-> yes

3. Are substantial conclusions reached?

-> yes partially

4. Are the scientific methods and assumptions valid and clearly outlined?

-> not really valid as stated above

5. Are the results sufficient to support the interpretations and conclusions?

-> not for some assumptions that are made in the context of carbohydrate excretion or Si-remineralisation

6. Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)?

-> yes

7. Do the authors give proper credit to related work and clearly indicate their own new/original contribution?

-> yes

8. Does the title clearly reflect the contents of the paper?

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7, C2465–C2469, 2010

-> yes

9. Does the abstract provide a concise and complete summary?

-> yes

10. Is the overall presentation well structured and clear?

-> partly according to the minor comments

11. Is the language fluent and precise?

-> yes

12. Are mathematical formulae, symbols, abbreviations, and units correctly defined and used?

-> yes

13. Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated?

-> yes see comments

14. Are the number and quality of references appropriate?

-> Figure 8 is not necessary

15. Is the amount and quality of supplementary material appropriate?

-> there is no supplementary material

Interactive comment on Biogeosciences Discuss., 7, 5953, 2010.