Biogeosciences Discuss., 6, S1179–S1188, 2009
www.biogeosciences-discuss.net/6/S1179/2009/

Biogeosciences Discussions

Interactive comment on "Bioavailability of organically bound Fe to model phytoplankton of the Southern Ocean" *by* C. S. Hassler and V. Schoemann

Anonymous Referee #1

Received and published: 24 June 2009

General Comments

This laboratory study examines the influence of organic ligands on iron (Fe) solubility and Fe bioavailability to Southern Ocean phytoplankton isolates. In general, the experiments are well conceived and executed. In particular, the experiments examining polysaccharide ligands as Fe sources are novel and merit publication. Similarly, the experiments examining the effects of these 'model' ligands on Fe solubility are potentially of great interest and relevance. However, I have a number of serious concerns regarding the design and interpretation of the short-term Fe and C uptake experiments, which I detail below. Provided these concerns are addressed I think this paper merits

publication as it will provide new insights into the processes governing Fe speciation and bioavailability.

Specific Comments

I have a number of questions regarding the design and interpretation of the Fe and C uptake experiments. I've particular concern with the author's use of short-term Fe and C uptake rates to infer the degree of Fe-limitation and/or Fe requirements of the strains they examined. Briefly reviewing the literature, steady-state Fe:C ratios are dependent on several factors including:

1) The amount of Fe available in the growth medium. Fe:C ratios increase with increasing Fe supply (e.g. Sunda and Huntsman 1995)

2) The provenance of the species. Oceanic species generally require lower Fe:C ratios to maintain maximum growth rates compared to coastal species (e.g. Sunda and Huntsman 1995, Maldonado and Price 1996)

3) Growth irradiance. Several studies have reported that the Fe requirements, and hence Fe:C ratios, increase with deceasing irradiance (e.g. Sunda and Huntsman 1997, Strzepek and Harrison 2004).

Additionally, short-term Fe uptake rates have been shown to be dependent on:

1) The photolability of the Fe-ligand complex (e.g. Maldonado et al. 2005)

2) The amount of Fe available in the uptake medium. Fe uptake rates increase with increasing Fe supply (e.g. Maldonado and Price 2000)

3) The degree of Fe-limitation experienced by the culture at the time the short-term incubation is conducted. There is evidence from an oceanic diatom that uptake of organically-complexed Fe is upregulated in Fe-limited cultures relative to Fe-replete cultures (e.g. Maldonado and Price 2001).

Regarding the design of the experiments, I have the following comments:

BGD

6, S1179–S1188, 2009

Interactive Comment

1) There appears to be no assessment of the physiological state of the cultures prior to measuring Fe and C uptake rates. It is currently impossible to know with the information provided in the Methods section whether the cultures were Fe-replete or experiencing some degree of Fe-limitation. Although the dissolved Fe concentration in the growth medium was relatively high (0.29 nmol L-1) compared to Southern Ocean surface waters, it is within the range reported to be Fe-limiting for some Southern Ocean species. Similarly, the natural ligands in the growth medium will affect Fe chemistry and bioavailability. Unfortunately, their concentrations and conditional stability constants are not reported. As the degree of Fe-limitation affects short-term uptake rates, it is crucial that the physiological state of the cultures is determined. This could be easily accomplished by reporting steady-state growth rates and, ideally, some other assessment of physiological state, such as Fv/Fm. In addition, these measures would need to be compared to those from a high Fe 'control' treatment. With the information currently available, there is no way to tell if the species examined were comparably Fe-stressed and hence what impact this may have on their short-term uptake rates.

2) The authors have chosen to compare two coastal isolates (Phaeocystis sp. and Chaetoceros sp.) with an oceanic isolate (F. kerguelensis) and a diatom isolated from coastal Norway. Given the differences observed between the Fe requirements of oceanic and coastal isolates, can the authors comment on how this may affect the interpretation of their results?

3) As the uptake experiments were conducted under illumination, it would seem important to discuss the photolability of the ligands used. Where any of the ligands photolabile? If so, how might that affect the rate of Fe uptake compared to photostabile Fe-ligand complexes (e.g. FeDFB, FeCAT)?

4) Similarly, why was Phaeocystis grown at 120 μmol photons m-2 s-1 and the other species at 60 μmol photons m-2 s-1? Given the difference in Fe requirements that may result from growing cultures under different irradiance regimes, this aspect of the experiment seems poorly controlled.

BGD

6, S1179–S1188, 2009

Interactive Comment

Regarding the interpretation of the uptake experiments, I have the following comments:

1) The authors state (p. 1692, line 5): 'Fe to carbon ratio is usually higher for Fe-replete as compared to Fe-limited cells (e.g. Twining et al., 2004). Based on the Fe:C observed for the control treatment (filltered Antarctic seawater), variable Fe limitation was experienced for the studied strains, with Fragilariopsis being the most limited with the lowest Fe:C ratio.' Firstly, Fe:C in this case usually increases within a species as Fe supply in the medium increases. Secondly, these experiments are conducted under steady-state (e.g. Sunda and Huntsman 1995) or quasi-steady-state conditions (Twining et al. 2004), rather than in short-term incubations. Given the number of factors that can affect Fe:C ratios, it is not possible to use this ratio to assess Fe limitation.

2) The authors state (p. 1963-1694): 'In contrast, with higher Fe:C ratios, Chaetoceros sp. and Phaeocystis sp., mainly present as solitary cells, did not appear strongly Fe-limited in our experiment. These strains should not be easily Fe-limited as a result of (i) high Fe diffusive supply because of their small size (as single cells), and higher surface to volume ratio (Pahlow et al., 1997), and (ii) lower Fe requirement as compared to the other studied strains (Coale et al., 2003; Timmermans et al., 2004).' As stated above, the Fe requirements of a species cannot be assessed solely on their short-term Fe:C uptake ratios. Furthermore, I am aware of no precedent to suggest that higher Fe:C ratios are indicative of lower Fe requirements. Finally, Coale et al. 2003 would not seem to be a very appropriate citation as they conclude that Phaeocystis has higher Fe requirements compared to diatoms, the opposite to what you state. Also, I'm assuming the paper you are referring to is 'Coale et al. (2003) Phytoplankton growth and biological response to iron and zinc addition in the Ross Sea and Antarctic Circumpolar Current along 170°W. DSR II 50: 635 -653.' This citation is not listed in the References.

I have several comments pertaining to the normalization of the short-term Fe and C uptake data. I am unclear as to why the authors chose to present data normalized per cell in Figure 1. Given that the organisms examined range in cell volume by several

BGD

6, S1179–S1188, 2009

orders of magnitude, it is not surprising that larger cells have higher rates of Fe uptake per cell. What is more revealing is the rate at which they take up Fe per unit cell volume (roughly equivalent to cellular demand) or per unit cell surface area. The authors more or less suggest in the Results and Discussion sections that normalizing the Fe uptake data to cell surface area is preferable. I would agree with this and therefore suggest presenting cell surface area-normalized uptake rates in Fig. 1, and present Fe uptake rates normalized per cell in Supplemental Table 1.

The second comment is specific to F. kerguelensis. Rather than normalizing rates per cell, or some derivation thereof, as was done for the other species, the authors have elected to normalize rates per chain of cells. Although I understand the reason for doing so, I do not necessarily agree with it. Although the cells are arranged in a chain, doesn't each cell contribute to the overall uptake rate? Furthermore, my personal experience with this species is that chain length is highly variable and can change considerably depending on the care with which the culture is handled during the growth and harvesting stages of an experiment. In contrast, the size of each cell is influenced primarily by growth conditions such as Fe availability. Finally, normalizing the data in this way overly complicates comparison amongst species. For these reasons, I would strongly suggest reporting the values of Fe and C uptake for F. kerguelensis normalized per cell, or more preferably to cell volume or cell surface area (see above).

1) Abstract: I don't think referring to the organisms you studied as 'keystone' species is justified. Do you have evidence that these particular species have a disproportionate effect on the Southern Ocean environment relative to their abundance? I believe a case could be argued for F. kerguelensis. However, 2 of the 4 organisms were not identified to the species level and one species, Thalassiosira antarctica, was isolated from Norway. It would have been more appropriate to examine a clone isolated from the Southern Ocean.

2) Abstract: For all strains studied, the bioavailability of Fe can be enhanced in presence of porphyrin, cathecolate and saccharides whereas it was decreased in presence

BGD

6, S1179–S1188, 2009

Interactive Comment

of hydroxamate and organic amine.

This statement is not supported by the data. The bioavailability of Fe was not enhanced relative to the inorganic Fe control in the presence of CAT, PIX, or sacccharides for Thalassiosira antarctica (Fig. 2). Similarly, CAT increased the bioavailability of Fe only for Chaetoceros sp.

2) Introduction: 'Therefore diatoms could be out-competed by cyanobacteria, when bioavailable Fe concentrations are limiting (e.g. Volker and Wolf-Gladrow, 1999).'

While this may be true of temperate and tropical regions, cyanobacteria are generally absent from Southern Ocean waters, presumably due to low temperature. You should state clearly that competition between these groups is likely negligible in Southern Ocean habitats.

3) Introduction: 'In fact, some neritic phytoplankton are able to specifically recognise Fe bound to siderophores (Trick et al., 1983).'

No subsequent studies have verified the findings of Trick et al., 1983. I suggest removing this passage.

4) Results: 'The presence of 1 nmol L-1 Fe or 1 nmol L-1 Fe with organic ligands did not statistically affect carbon fixation. Carbon cellular uptake rate were similar following both 2 h and 16 h incubations.'

You should state the level of statistical significance. Again, stating the results were similar is vague. Why are the results of the 16 h incubation not reported? I would argue that these results are more appropriate to compare to the Fe uptake data, which were also measured during 16 h incubations. I would strongly suggest that you report the carbon uptake rates from both the 2 and 16 h incubations, including a full statistical analysis.

5) Discussion: 'Although higher Fe:C was previously reported for picoplankton as compared to larger phytoplankton in subantarctic waters (McKay et al., 2005)'

BGD

6, S1179–S1188, 2009

Twining et al. 2004 also report higher Fe:C ratios for small phytoplankton compared to larger phytoplankton in Southern Ocean waters.

6) Discussion: 'Under these circumstances, Fragilariopsis would slowly respond to changes in environmental conditions and long-term, sustained, Fe enrichment might be required for Fragilariopsis to bloom.'

A Fragilariopsis kerguelensis bloom developed rapidly following two short-term Fe additions during the SOIREE mesoscale Fe enrichment experiment (Boyd et al. 2000). This would appear to refute the author's interpretation. More to the point, the entire interpretation of the Feext:Feint ratio assumes that the extracellular Fe is 'associated with extracellular binding sites', rather than passively sorbed to the cell surface. How can the two processes be distinguished? If they cannot be, what implication does that have for the interpretation of the Feext:Feint ratio?

7) Discussion: 'In order to enhance Fe bioaccumulation, the complex needs to be taken up directly or to be specifically bioavailable (such as siderophores).

I'm not sure I understand this statement. By 'specifically bioavailable' do you mean that cell must have a receptor capable of recognizing the Fe-siderophore complex? This need not be the case if eukaryotic phytoplankton possess a non-specific extracellular reductive mechanism to liberate Fe from ligand complexes. Could you please clarify this statement?

8) Discussion: 'The presence of 1 nmol L-1 Fe and 15 nmol L-1 PIX resulted in an enhanced Fe bioaccumulation as compared to the 1 nmol L-1 Fe enrichment for the four phytoplankton studied, in accordance with what was previously reported for polar eukaryotic phytoplankton (Hutchins et al., 1999).'

No polar phytoplankton were examined by Hutchins et al. (1999).

Technical corrections

1) Abstract: 'The effect of excess of

BGD

6, S1179–S1188, 2009

Interactive Comment

Remove the second 'of'.

2) Abstract: 'For all strains studied, the bioavailability of Fe can be enhanced in the presence of porphyrin, cathecolate and saccharides whereas it was decreased in presence of hydroxamate and organic amine.'

Enhanced or decreased relative to what? Presumably relative to the inorganic Fe treatment.

'Cathecolate' and 'hydroxamate' should be changed to 'a catecholate siderophore' and 'a hydroxamate siderophore', respectively (note the corrected spelling of 'catecholate').

Insert 'the' between 'in' and 'presence'.

3) Introduction: 'A fraction (viz. the bioavailable fraction) of this Fe is expected to be accessible for phytoplankton growth, therefore controlling the plankton abundance and diversity (e.g. de Baar et al., 2005)'

Delete 'therefore controlling the plankton abundance and diversity' - it is redundant.

4) Reference for the citation 'Mancuso et al., 2005' is missing.

5) Introduction: 'Both biological processes are known to be Fe responsive (e.g. Kirchman et al., 2000; van Oijen et al., 2005).'

This sentence does not follow from the preceding sentence. Which processes are you referring to? Presumably, these processes are carbon storage by phytoplankton and bacterial respiration.

6) Materials and Methods: 'Calculated cellular biovolume were similar to the volume determined by Coulter counter for Phaeocystis, Chaetoceros and Thalassiosira.'

Stating the two techniques yielded 'similar' results is vague. It would be preferable if the results were reported to be statistically insignificant (including the level of significance), if this is the case.

BGD

6, S1179–S1188, 2009

7) Results: 'Phaeocystis has the highest C uptake per unit of cell which can be only partly due to the carbon allocated to the production of mucus in the colonies since most of the cells are isolated in the experimental conditions'

I would suggest changing 'most of the cells are isolated in the experimental conditions' to 'cells were predominantly in the solitary form under our experimental conditions'.

References

Boyd PW, Watson AJ, Law CS, Abraham ER, Trull T, Murdoch R, Bakker DCE, Bowie AR, Buesseler KO, Chang H, Charette M, Croot P, Downing K, Frew R, Gall M, Hadfield M, Hall J, Harvey M, Jameson G, LaRoche J, Liddicoat M, Ling R, Maldonado MT, McKay RM, Nodder S, Pickmere S, Pridmore R, Rintoul S, Safi K, Sutton P, Strzepek R, Tanneberger K, Turner S, Waite A, Zeldis J (2000) A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature 407: 695-702

Maldonado MT, Price NM (1996) Influence of N substrate on Fe Requirements of Marine Centric Diatoms. Mar Ecol Prog Ser 141: 161-172

Maldonado MT, Price NM (2000) Nitrate regulation of Fe reduction and transport by Fe-limited Thalassioisira oceanica. Limnol Oceanogr 45: 814-826

Maldonado MT, Price NM (2001) Reduction and transport of organically bound iron by Thalassiosira oceanica (Bacillariophyceae). J Phycol 37: 298-309

Maldonado, MT, Strzepek, RF, Sander, S, and Boyd, PW. (2005) Acquisition of iron bound to strong organic complexes, with different Fe binding groups and photochemical reactivities by plankton communities in Fe-limited subantarctic waters, Global Biogeochem. Cycles, 19, GB4S23, doi:10.1029/2005GB002481

Strzepek, RF, Harrison PJ (2004) Photosynthetic architecture differs in coastal and oceanic diatoms. Nature 431: 689-692

Sunda WG, Huntsman SA (1995) Iron uptake and growth limitation in oceanic and

BGD

6, S1179–S1188, 2009

coastal phytoplankton. Mar Chem 50: 189-206

Sunda WG, Huntsman SA (1997) Interrelated influence of iron, light and cell size on marine phytoplankton growth. Nature 390: 389-392

Interactive comment on Biogeosciences Discuss., 6, 1677, 2009.