Biogeosciences Discuss., 4, S539–S544, 2007
www.biogeosciences-discuss.net/4/S539/2007/

Biogeosciences Discussions

Interactive comment on "Carbon isotope fractionation in developing natural phototrophic biofilms" *by* M. Staal et al.

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Received and published: 22 May 2007

We think that reviewer #1 had many valuable comments and questions and based on his review report and the editors report we can supply some additional information As suggested by the editor we will change the title, and we propose as a title: "Differences in carbon isotope fractionation patterns during the development of phototrophic freshwater and marine biofilms" .

Incubator design and experimental setup.

Concerning the questions about the incubator, we will add the following additional information on the incubator design in the revised ms: The incubator holds 4 lanes and every lane had a specific light intensity. Every lane had its own medium reservoir and the medium in these reservoirs was refreshed twice a week. The water height above

the slides (or biomass) was approximately 2-3 mm, resulting in flow rates of $\sim$0.5 and $\sim$2 m/s for the 25 and 100 L/h runs, respectively, indicating thin diffusive boundary layers (for more specific comments on the diffusive boundary layer, see below). We will include this information in the methods session.

Both the editor and reviewer #1 commented on the lack of replication for growing the biofilms. In total five incubators were operated simultaneously at different locations by different research groups, with approximately the same results, though with some difference in the lag times in the biofilm growth patterns. It was found that the trends in the maximal growth rates with irradiance were approximately equal for the different incubators, but the population structure differed (Roeselers et al, 2006). We believe that comparison of these growth curves of the different incubators is a study on itself and does not necessarily lead to a better insight in relation to the present study, since no δ^{13}C measurements were performed on the other biofilm incubators.

One comment of both reviewer#1 and the editor concerns the estimation of the growth rates. The fit quality of the growth curve was good (average r^2=0.975 $\pm$0.038, the lowest r^2 value was 0.820).

Theoretically it can be argued that net growth will stop at a certain point due to light limitation, where the depth integrated photosynthesis rate will reach a maximum and will be balanced due to an increase in consumption/respiration per m^2 due to the thickening of the biofilm. The model presented in Wolf et al, 2007 (which was the same model as used in this ms) also shows that the biofilm cannot exhibit unlimited growth to an infinite thickness. We checked our data and by plotting the natural logarithm of the absorption vs time we could observe that that the growth rate indeed decreases above 50-70% light absorption to virtually zero at $>$85%. In addition, we estimated for one run the growth rate in the initial phase (Linear fit in the ln graph), and found exactly the same trend in growth rate as was found with the logistic growth model. We admit that indeed a ln graph as discussed above may visualizes the argument put forward in our ms. However, we think this information can be considered more or less as text book

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knowledge and is not really novel scientific information and may be not necessary for our ms. But the graph can be included in the ms, if the editor thinks it strengthens the ms.

Concerning the suggestion to plot δ^{13}C values vs. Growth rate we can say that there is a problem with estimating the growth rate at high light absorption levels. One reason for this is that the linear relationship between light absorption and biofilm biomass (wet weight) is lost above 85% light absorption due to an increase in EPS formation. That is why we think it is better to plot δ^{13}C values versus absorption. An additional point of consideration is that the new biomass at these low net growth conditions are only adding little to the existing biomass. Therefore, there will be no correlation with growth rate and the measured δ^{13}C values. There will only be a correlation between actual growth rate and fractionation level, since that parameter corrects for the history of the biomass.

Since irradiance was the only varied parameter per treatment, we do not understand why reviewer #1 can not see that, despite the big differences in maximum relative growth rate between the different irradiances in the initial and exponential growth phase (growth rate was mostly 4 times higher at the high irradiances relative to the low irradiance), this did not result in differences in the δ^{13}C values. Figure 2 and 3 of the ms clearly show that very little variation was present in the δ^{13}C values during the initial phase. Differences in δ^{13}C (relative to the initial value) only developed with the thickening of the biofilm.

Statistical considerations

We agree that the experimental design was not optimal to define the specific contribution of every individual parameter to the changes of the δ^{13}C values. However, we can not change anything on the experimental design anymore and we can not perform additional runs. This means that we have to work with the data available thus far and we think, as was also clearly indicated by the reviewer that our findings are worth

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publishing.

The initial purpose of the experimental set up was to gather as much as possible data for the phototrophic biofilm growth model. For us it was just very surprising to find that the trends in δ^{13}C values were so similar within one media type, despite the differences in treatments. Instead of using statistics to explain the contribution of some parameters, we rather used the mathematical model to explain the trends found. This is to our opinion a valid and useful approach, since it will lead to a fundamental understanding of the biogeochemical processes that play a role in fractionation during biofilm development.

There were another reason, more practical reason for the experiment design. Since we were measuring PLFA, pigment composition, photosynthesis and δ^{13}C values per phase, and we needed a slide for each type of measurements we simply had no extra slides left. We like to add that we did perform one triplicate measurement for δ^{13}C values in a fresh water test run, to test whether one slide could cover heterogeneity of the total system. The δ^{13}C values were (n=3 slides), -23.333$\pm$0.478, -24.546$\pm$0.333, -27.66$\pm$1.074, -35.172$\pm$0.0165 for the lane with respectively 120, 60, 30 and 15 μmol photons m^{-2} s^{-1} irradiance. These data will be included in a revised version.

We would also like to point out that one run lasted over 40 days, so the whole series of experiments took over 1.7 years (including three test runs). Within the project we only could use the incubator for a period of less than 2 years, so there was no room for repetition of an experiment. Repetition seems impossible anyhow, since the inoculate we used to seed the biofilms were young (within three days after harvesting we used the innoculum), and the composition of the biofilms used for the inoccullum would vary with the season. Long term storage of the innoculum was found to affect the initial growth rate. In addition it was found that the population structure was different, even when seeded from the same innocculum at the same time, but in differ different incubators (Roeselers et al. 2006).

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We checked some additional data, and we can say that we found a similar trend in δ^{13}C values with the development of freshwater biofilms (biomass measured as wet weight in this case) in an helophyte filter placed behind a waste water treatment. These biofilms have been grown at three depths (three different light intensities) and have been sampled twice (after one week and after two weeks). We can include this data in the ms if the editor thinks it is necessary.

Effect of flow on δ13C and heterotrophic activity. Effect of flow rate: the flow rate of 0.5 and 2 m/s apparently did not affect the mass transfer enough to result in differences in growth rate. As could be deduced from fig 6, the diffusive boundary layer at 2 m/s seems to be 50-100 μm thick, while the DBL was 50-150μm thick in the 0.5 m/s runs. The diffusive boundary layers are thin at both flow regimes so no big effect of the DBL are to be expected. Therefore, it seems rather unlikely that the low fractionation levels in the initial phase are the result of the low flow velocity as suggested by reviewer#1.

Reviewer#1 did not give a good reason why external mass transfer is unimportant while internal mass transfer is important. One reason to consider external mass transfer important is that per se the net growth of a biofilm is linked to external mass transfer. Since net growth is the most important process in the initial and exponential growth phase, it seems that during these phases external mass transport must be important as well. During especially the initial phase, limitation of external mass transport may be absent. However, with the thickening of the biomass, such limitations will become more important.

In addition, heterotrophic activity and internal mass transfer seems intimately connected, since internal inorganic C is produced by heterotrophic organisms. All inorganic carbon will be consumed under C-limited conditions indicating that the respiratory rate will determine recycling rates, rather than the length of the diffusion pathway since the heterotrophic biomass will also be present in the photic zone of the biofilm. Therefore, we do not see a good reason to implement a “third” factor (which may be considered as the internal mass transfer limitation) in the discussion.

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Conclusion

We think that our conclusion that the δ^{13}C values of the biofilms are dependent on the net areal photosynthesis rate rather than on the growth rate is rather well argued for, and seems to be backed up by the model of the freshwater biofilm (Wolf et al, in press). We would like to point out that similar results have just been published in an article of Cornelisen et al (2007) in the last issue of L&O. They found that the patterns in the δ^{13}C of *Ulva pertusa* depended mostly on the interaction between net photosynthesis rate as well as on the nutrient source pools (CO_2 vs HCO_2) and only little on the flow rate. This is also our conclusion for the δ^{13}C patterns observed in the biofilms. However, we will change the text in the conclusion section in order to make them more clear and concise.

References

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