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Methane production by three widespread marine phytoplankton species: release rates, precursor compounds, and potential relevance for the environment

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Supplementary

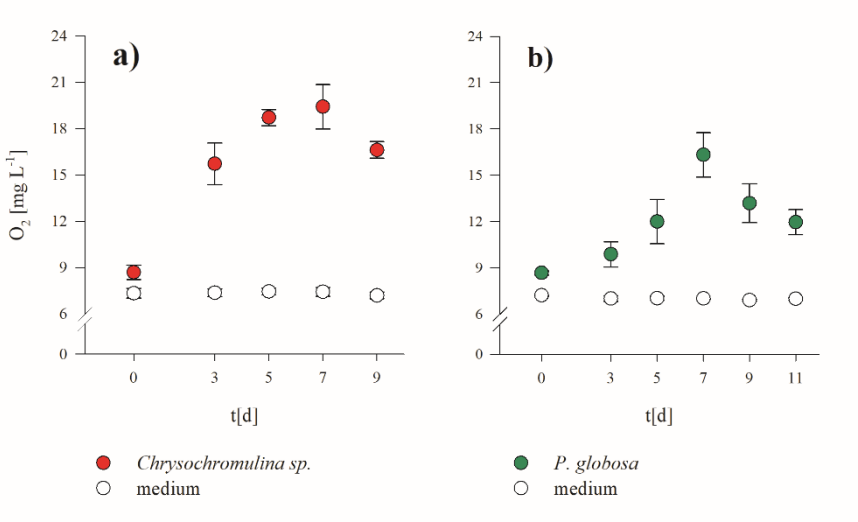

Fig. S1: Dissolved oxygen concentration in course of time from algae species *Chrysochromulina sp*. and *P. globosa*. Mean values of six replicated culture experiments are shown and error bars mark the SD.

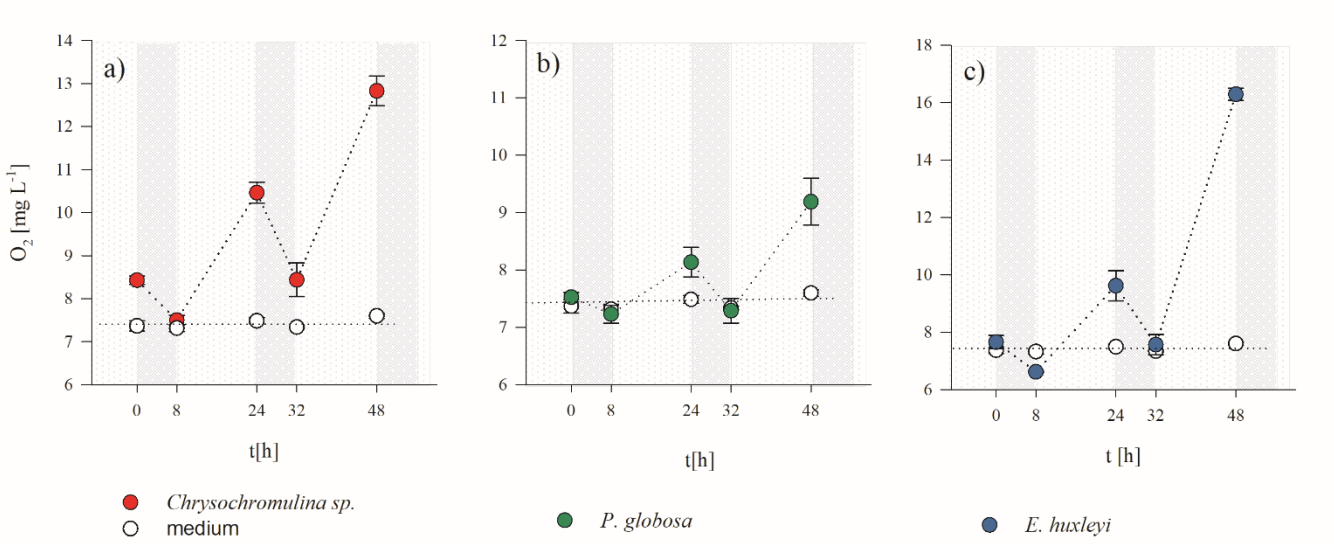

Fig. S2: Dissolved oxygen concentration in dark and light periods of three algae species *Chrysochromulina sp.*, *P. globosa* and *E. huxleyi*. Mean values of four replicated culture experiments are shown and error bars mark the SD. White and grey shading mark the light and dark periods.

S3 Further Discussion regarding the interplay between algae and bacteria: Bacteria might also contribute to the observed CH_4 production, but even if they did they still would depend on algal growth in our cultures as demonstrated by the following reasons:

1) <u>The CH_4 production rates decreased with decreasing algal growth rates:</u>

In batch cultures, the algae cultures undergo various stages of growth (see section 3.1, Fig. 3 a-c). Bacterial density increases tremendously when algae culture reach stationary growth phase and excretion of organic products from senescent alga cells together with the decomposition of cells is providing substratum for heterotrophs. This was described in literature (Salvesen et al., 2000) and is in line with our own experience with growing alga cultures in batch mode. In section 3.1 cultures have undergone transitionary growth phase leading up to the stationary phase. We calculated daily incremental CH_4 production rates (not shown in the manuscript). The CH_4 production rates of

each species decreased with decreasing growth rates and decreased drastically when approaching stationary phase. This observation is the opposite of what we would have expected, if CH_4 were mainly produced by bacteria. It would however be compatible with the idea that algae produce precursors which are subsequently used by bacteria to produce CH_4.

2) Light is a prerequisite for CH_4 formation in algae cultures:

Cultures of *E. huxleyi,* and *P. globose* were incubated under a day-night-cycle and continuous darkness. Methane concentrations did not increase when cultures were incubated in darkness while concentrations increased in cultures growing under a day-night-cycle. This is a strong indication that CH_4 formation is dependent on the light-dependent metabolism of the algae, since the metabolism of heterotrophs or archaea is independent of light. While the latter conclusion does not rule out the "algae precursor scenario", our experimental setup makes it rather unlikely. In these experiments we inoculated high cell densities ($\approx 10^5$ cells mL^{-1}) because they were designed to be short term which requires a high start cell density to yield measurable production. Therefore the start conditions will have included a seawater replete with precursors. It is unlikely that the relatively few bacteria present should have become precursor-limited over a single dark phase. It is rather more likely that the pool of precursors was sufficient to sustain bacterial CH_4 production over the dark phase. In this scenario an extra precursor production by cultures exposed to light would have been without effect on CH_4 production.

3) It is highly unlikely that methanogenic ***archaea*** are the source of CH_4 in cultures where CH_4 is produced alongside oxygen (incubation under day-night-cycle).

If archaea were the CH_4 source we would have expected a higher CH_4 production in the dark.

4) Selectively inhibition of algal growth reduced CH_4 production rates:

We compared emission rates of *E. huxleyi* that have been treated with and without 3-(3,4 dichlorophenyl)-1,1-dimethyl-urea (DCMU). DCMU acts as an inhibitor of photosynthesis (Wessels and Van Der Veen, 1956). Selectively inhibition of algal photosynthesis reduced both algal growth rate and CH_4 production rates. In the inhibition experiments, the growth rate was only 29% of the uninhibited culture and the CH_4 production rate dropped to 18% of the uninhibited culture. Since the inhibition effect of DCMU is very selective for algae (Francoeur et al., 2007) the result may indicate direct CH_4 production from algae.

Although we regard it as unlikely, we cannot strictly rule out the "precursor-scenario": Bacteria use algae-derived precursors to produce CH_4, and these bacteria require constant production of these precursors by algae. In other words, the precursor-production by algae is the rate limiting step of CH_4 production by bacteria (as evident from points 1, 2, and 4 above). If true the CH_4 production observed in our experiments would be the result of a "collaborative effort" which needs both partners, algae and bacteria. This would be a significant finding and prompt further research. Questions to be addressed would include: what are the precursors? Which algae can produce the precursors? Which bacteria can produce CH_4 using these precursors? Is it possible to grow the respective algae without the bacteria (not all algal cultures can survive in an axenic state). Can the same CH_4 production be achieved by growing the bacteria without algae and adding the precursors? This selection of questions would suffice for an entire research project. Meanwhile we are content with describing CH_4 production that depends on algae, whether solely or in cooperation with bacteria.

To sum up, our ***main finding*** is that CH_4 production in ***mixed algae/bacteria cultures*** depends on ***algal growth*** and is not supported when algae become senescent. Future research will clarify whether algae alone produce CH_4 or whether they produce precursors which in turn are used by bacteria to produce CH_4.

References

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