

## ***Interactive comment on “Nitrous oxide (N<sub>2</sub>O) production in axenic *Chlorella vulgaris* cultures: evidence, putative pathways, and potential environmental impacts” by B. Guieysse et al.***

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It is indeed interesting that although this ability of microalgae has been discussed for decades (Cohen and Gordon, 1978) and was partially confirmed in controlled cultures in 1984, its significance seems to have been gradually lost until very recently. The relevance of this study must therefore be assessed in regards to the lack of knowledge about this potential issue in the algae biotechnology field and the massive investments that have been poured into the development of algae biotechnologies in the last 5 years.

Global significance: As of today, the microalgae biotechnology industry is most likely

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too small to have any significant impact on the global N<sub>2</sub>O cycle (we do not speculate, however, on the potential significance of algal N<sub>2</sub>O emissions from natural ecosystems). Hence, we have a unique opportunity to raise awareness and tackle this potential issue before it becomes globally significant.

The significance of these emissions must be evaluated within the context of recent massive investments to develop new capacities for mass algae production. Thus, and based on the N<sub>2</sub>O specific production rates reported outdoors in the absence of external nitrite (4.4 – 32.3 nmol g DW<sup>-1</sup> h<sup>-1</sup>), a 0.25 m deep high rate algae pond operated at 7-days HRT under Mediterranean climate could release 1.38 - 10.1 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>. If we then assume that 80% of the energy found in the biomass-lipids is recovered as biofuel (e.g. 20% of the total biochemical energy photosynthesized), the GHG equivalent of N<sub>2</sub>O accounts for 1.96 – 14.4 g CO<sub>2</sub>-equivalent MJ<sup>-1</sup> fuel. To provide an alternate metric, these emissions are equivalent to 1.3 - 31.5 ton CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup> for a production of approx. 68 ton algae-DW ha<sup>-1</sup> yr<sup>-1</sup>. To put these numbers into perspective, the commercialization of algae fuels would likely be associated with the production of millions of tons of algae biomass each year.

We must also remind that N<sub>2</sub>O is not merely a greenhouse gas; it is also a powerful ozone depleting pollutant.

Strategies for mitigation: Based on the batch assays, the use of ammonium as sole N-source has the potential to mitigate N<sub>2</sub>O emissions from axenic *Chlorella vulgaris* culture. This was confirmed in continuous cultures (manuscript under preparation). However, we cannot speculate for other algae species and it is still possible bacterial or archaeal nitrifiers may generate significant N<sub>2</sub>O emissions from non-axenic cultures supplied with ammonium as N-source.

We have observed that not all algae release N<sub>2</sub>O under the experimental conditions described in the paper (unpublished data). Algae selection is therefore another mitigation option.

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NR-activity: We did not verify NR activity in vitro but this enzyme and its ability to reduce nitrite is well described in the literature. The effect of the inhibitor was shown in vivo as described in the manuscript. We agree that in vitro confirmation would be helpful.

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