

# Membrane Inlet Mass Spectrometry at the Crossroads of Photosynthesis, Biofuel, and Climate Research<sup>1[OPEN]</sup>

Dear Editor,

Microalgae are continuously shaping Earth's atmosphere through oxygenic photosynthesis, and nowadays, half of the photosynthesis is attributed to microbial photosynthesis (Field et al., 1998; Behrenfeld et al., 2005). While algal photosynthesis contributes to offsetting the CO<sub>2</sub> footprint, it also produces nitric oxide (N<sub>2</sub>O), a potent greenhouse gas. In some ecological niches microalgae can produce hydrogen (H<sub>2</sub>), a promising energy carrier; therefore microalgae are actively explored for their potential as a platform for production of renewable energy. Measuring gas exchange between algae and the atmosphere, and understanding biological mechanisms underlying photosynthetic CO<sub>2</sub> capture, and O<sub>2</sub>, H<sub>2</sub>, or N<sub>2</sub>O production, holds great promise—not only to better evaluate the reciprocal effects of global changes on oceanic carbon sinks, but also to explore the limits of biomass and biofuel productivity of algae. Membrane inlet mass spectrometry (MIMS) was initially developed to measure O<sub>2</sub> exchange between algal cells and the surrounding liquid medium (Hoch and Kok, 1963), and its use has since been extended to other gases including H<sub>2</sub> and more recently, N<sub>2</sub>O (Burlacot et al., 2020). Here we review recent breakthroughs allowed by MIMS in dissecting molecular mechanisms of gas exchange in microalgae (Fig. 1) and provide perspectives on how MIMS will be crucial to address future challenges in algal research.

## DECIPHERING PHOTOSYNTHETIC OXYGEN UPTAKE MECHANISMS

MIMS was initially developed to monitor O<sub>2</sub>-consuming processes during photosynthesis (Hoch and Kok, 1963). The use of <sup>18</sup>O labeling made it possible to differentiate O<sub>2</sub>-consuming processes from O<sub>2</sub>-generating processes. While O<sub>2</sub> is produced from water splitting by PSII, various mechanisms participate in O<sub>2</sub> consumption including mitochondrial respiration, chlororespiration, PSI-driven reduction, and photorespiration (Curien et al., 2016). Measuring O<sub>2</sub> exchange by MIMS has contributed

to the discovery of several factors involved in O<sub>2</sub> consumption, including the plastid terminal oxidase (Cournac et al., 2000) and flavodiiron proteins (FLVs; Helman et al., 2003). Recently, the use of MIMS has also helped to reveal the occurrence of redox communication between mitochondria and chloroplasts (Dang et al., 2014; Bailleul et al., 2015), and between chloroplasts and peroxisomes (Kong et al., 2018). Intriguingly, although FLVs are recognized as major factors in light-dependent O<sub>2</sub> uptake in cyanobacteria (Helman et al., 2003; Allahverdiyeva et al., 2013; Santana-Sanchez et al., 2019) and green microalgae (Chaux et al., 2017), they are absent from diatoms where interaction between chloroplasts and mitochondria is critical (Bailleul et al., 2015; Flori et al., 2017). The use of MIMS will be crucial in establishing the significance of such diverse bioenergetic mechanisms among algal species in relation to their ecological niches and environmental constraints.

## MEASURING CO<sub>2</sub> GAS EXCHANGE AND THE CARBON-CONCENTRATING MECHANISM

Many microalgae and cyanobacteria have the ability to improve the affinity of photosynthesis for dissolved inorganic carbon (DIC) when grown at limiting DIC levels (Badger et al., 1980; Reinfelder, 2011). This property is due to the induction of a carbon-concentrating mechanism, which involves, among other components, active bicarbonate pumping and carbonic anhydrases (Mackinder et al., 2017; Mackinder, 2018). MIMS has been used to elucidate different aspects of carbon-concentrating mechanisms, including (1) the disequilibrium between bicarbonate and CO<sub>2</sub> pools resulting from this active pumping (Badger and Price, 1989; Sültemeyer and Rinast, 1996; Sültemeyer et al., 1998), (2) *in vivo* activity of carbonic anhydrases by following <sup>18</sup>O exchange between CO<sub>2</sub> and water (Tansik et al., 2015; Tolleter et al., 2017), and (3) more recently, the direct assessment of apparent affinity of photosynthesis for DIC (Douchi et al., 2019).

## MIMS AND BIOFUEL RESEARCH

The study of microalgae has been greatly boosted by biofuel research, as microalgae naturally produce energy-rich compounds such as H<sub>2</sub>, reserve lipids, or hydrocarbons (Stephens et al., 2010). Secretion of H<sub>2</sub> or short-chain hydrocarbons by photosynthetic cells avoids energy-costly steps of harvesting and extraction. H<sub>2</sub> is produced by microalgae through a coupling between hydrogenase (H<sub>2</sub>ase) and the photosynthetic electron transport chain.

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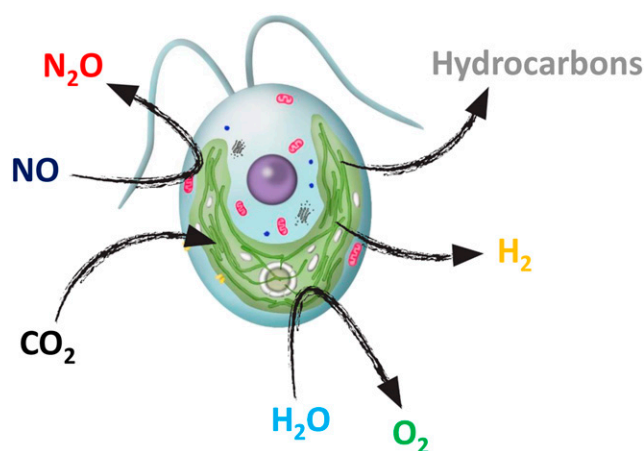
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**Figure 1.** Schematic view of gas exchange between microalgae and its surrounding medium illustrated in the model species *C. reinhardtii*.

By in situ-monitoring of  $H_2$  exchange performed in a time-resolved manner, MIMS contributed to the identification of biological bottlenecks of  $H_2$  production. These include the photosynthetic control triggered by the Proton Gradient Regulatory protein Like-1-mediated cyclic electron flow (Tolletier et al., 2011), competition for electrons with the Calvin cycle (Milrad et al., 2018), and competition with the FLV-mediated  $O_2$  photoreduction (Burlacot et al., 2018). Lately, MIMS has been used in the development of a very promising method for  $H_2$  photoproduction based on the intermittent illumination of microalgae (Kosourov et al., 2018; Jokel et al., 2019).

MIMS has also contributed to the study of hydrocarbon synthesis by microalgae. It enabled the detection of  $CO_2$  produced by the Fatty-Acid Photodecarboxylase (FAP), therefore demonstrating its decarboxylase activity and further establishing that FAP is a photoenzyme using blue photons as substrates (Sorigué et al., 2017). Taken together, MIMS should be useful in future studies aimed at characterizing the bottlenecks of volatile compound production by FAPs and  $H_2$ ase at the both enzyme and cellular levels.

### PHOTOREDUCTION OF NO INTO $N_2O$

$N_2O$  is a potent greenhouse gas responsible for 6% of the Earth's radiative force although it is present at a concentration 1,000 times lower than that of  $CO_2$  (IPCC, 2013). Recently,  $N_2O$  production by the photosynthetic chain has been demonstrated using real-time MIMS measurements in axenic cultures of *Chlamydomonas reinhardtii* (Burlacot et al., 2020). NO was shown to be produced during nitrogen assimilation and reduced into  $N_2O$  by FLVs in a light-dependent manner (Burlacot et al., 2020). These results open new perspectives for the study of mechanisms regulating the intracellular concentration of NO, an important signaling molecule in plants (Farnese et al., 2016). They also provide insights into the possible consequences of

algal blooms and large-scale algae cultivation on global warming.

### ADVANTAGES AND DRAWBACKS

When compared to other methods like gas chromatography or specific gas electrodes, MIMS has the great advantage of simultaneously measuring the concentrations of various gases in a time-resolved manner by selecting mass peaks specific to the gases of interest. This flexibility largely explains the recent success of MIMS (Ketola and Lauritsen, 2016), but it must be kept in mind that MIMS is demanding from a technical point of view (Shevela et al., 2018). Proper use of MIMS requires a suitable experimental setup (including efficient temperature control and limitation of gas leaks), as well as appropriate calibration and data processing procedures (Bailleul et al., 2017). The choice of the type of membrane, in particular its material (usually silicon or polytetrafluoroethylene) and thickness, is critical because the membrane permeability and selectivity affects the sensitivity of the setup and the relative enrichment of specific gases (Beckmann et al., 2009). Furthermore, the diffusion of gases through the membrane from the liquid sample to the vacuum pump of the mass spectrometer creates a gas consumption that needs to be corrected to determine gas exchange rates related to biological processes (Kotiaho and Lauritsen, 2002; Bailleul et al., 2017). Depending on the targeted application, a compromise must be found among membrane permeability (which affects both sensitivity and gas consumption), cell or enzyme concentration (which affects rates of gas exchange), and the volume of the reaction vessel (which affects gas consumption kinetics). Ultimately, the sensitivity of the MIMS is generally limited by (1) the permeability of the membrane and (2) gas leakage of the setup, which can reduce the use of the technique in highly diluted samples, such as found in the natural environment (Chatton et al., 2017).

### PERSPECTIVES

This decade has seen an increasing use of MIMS in addressing biological questions in algal research. This has helped to push the limits of the types of biological questions that we can address in photosynthetic microorganisms at various scales. Coupling MIMS measurements with chlorophyll fluorescence has proven to be of interest to study gas exchange beyond the theoretical limits of MIMS (Burlacot et al., 2018) and should allow deeper understanding of photosynthesis. Further coupling to absorption change measurements (Bailleul et al., 2010) should provide new insights into mechanisms regulating photosynthesis and their interactions in response to environmental constraints. While several  $O_2$  photoreduction mechanisms have been observed in algae (Curien et al., 2016), their physiological significance and their relevance in natural environments

remain largely unexplored. Recently, the miniaturization of MIMS allowed measuring in situ O<sub>2</sub> exchange rates in samples from the north Pacific (Ferrón et al., 2016) or in planktonic blooms from the north Atlantic (Bailleul et al., 2017), starting thus a new era for expanding our laboratory models to natural environments.

MIMS has now emerged as a key technique to study cellular mechanisms involved in the exchange of gas molecules with the medium, paving the way toward better understanding of the interaction among algae, aquatic ecosystems, and the atmosphere. In situ measurements of O<sub>2</sub> and CO<sub>2</sub> exchange in oceanic samples will provide a better understanding of the physiological relevance of mechanisms regulating photosynthesis under natural conditions. In another perspective, it will be of great interest to uncover the distribution of recently discovered N<sub>2</sub>O production pathways in aquatic ecosystems (Burlacot et al., 2020) and further estimate their global influence on the climate through measuring N<sub>2</sub>O production in oceanic hotspots.

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