

**Title:**

Enhancing Methanogenesis Performance in the Power-to-Methane process: Addressing H<sub>2</sub> and CO<sub>2</sub> Intermittency

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**Abstract (300 words maximum): :**

**Aims:** Power-to-Methane (PtM) technology stands at the forefront as a promising solution for the storage of surplus renewable energy aligned with CO<sub>2</sub> reduction. In the biological PtM process, methanogens utilize H<sub>2</sub> generated from water electrolysis powered by renewable energy and CO<sub>2</sub> from industrial waste gas to produce CH<sub>4</sub>, namely methanogenesis. Our work aims to enhance the robustness of PtM processes by optimizing this methanogenesis step, addressing challenges related to intermittent gas feeding.

**Methods:** The model strain *Methanococcus maripaludis* MM901 was cultured in batch cultures to study its starvation-revival dynamics under H<sub>2</sub>- vs. CO<sub>2</sub>- starvation by monitoring OD<sub>600nm</sub> and specific CH<sub>4</sub> production rate. To obtain mechanistic understanding of the observed difference, we also (1) measured NAD<sup>+</sup> and NADH in the cells during starvation using bioluminescent assays, as well as (2) performed oxygen exposure experiments, where cultures were exposed to air for 1h during starvation followed by monitoring their revival dynamics after O<sub>2</sub> removal.

**Results:** *M. maripaludis* MM901 exhibited a shorter lag time and higher metabolic activity after H<sub>2</sub> starvation compared to CO<sub>2</sub> starvation, and an increase in starvation time amplified the difference. We hypothesize that cells experience a reductive stress under CO<sub>2</sub> starvation. We measured NAD<sup>+</sup> and NADH in cells during starvation and observed significantly higher NADH/NAD<sup>+</sup> ratio in CO<sub>2</sub> starved cells indicating accumulation of reducing equivalence. O<sub>2</sub> exposure also exerts greater inhibition on the revival of CO<sub>2</sub>-starved cultures over H<sub>2</sub>-starved cultures shown by a significantly longer lag phase.

**Conclusions:** In CO<sub>2</sub>-starved cells where there is H<sub>2</sub> leftover, it is possible that O<sub>2</sub> reacts with accumulated reducing equivalence, resulting in the production of highly oxidative reactive oxygen species, and subsequently damaged enzymes and DNA in cells. To ensure the process stability and gas product quality, where H<sub>2</sub> leftover is preferred over CO<sub>2</sub> leftover, further efforts are needed to adapt methanogens to starvation under CO<sub>2</sub> limitation.