A Step Towards Understanding Electromethanogenesis

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Abstract

Electromethanogenesis is a process in which reducing equivalents in the form of an electrical current are supplied to a methanogenic culture to enable methane production from the reduction of carbon dioxide (CO_2) (1). Through electromethanogenesis, biogas could be upgraded to natural gas using renewable electricity. However, the underlying molecular mechanisms of electromethanogenesis remain elusive, as successful attempts have been mostly made with mixed cultures (2)(3) and with hydrogenotrophic methanogens (4). It was suggested that at potentials of -400 mV (vs. SHE) hydrogen is insufficient to sustain hydrogenotrophic growth, and therefore even hydrogenotrophic methanogens grow by direct electron uptake and therefore electromethanogenesis. Moreover, direct external electron transfer (DEET) from cathode to methanogens is considered a possibility because recent studies with cytochrome-containing methanogens revealed that even non-hydrogenotrophic methanogens could receive electrons directly from an electrogenic microorganism – *Geobacter metallireducens* (5).

We embarked upon discovering whereas H_2 is indeed not evolved at the electrode surface when cathodes are poised -400 mV (vs. SHE). We also tested a non-hydrogenotrophic methanogen, *M.horonobensis (MH)*, on the cathode at -400 mV, but also in co-cultures with the electrogen, *G. metallireducens (GM)* to determine if it has electromethanogenic properties. We learned that MH likely grew via electromethanogenesis. Next, we will investigate gene expression in MH/GM co-cultures as well as that of MH on electrodes to uncover the molecular mechanisms of strict direct electron uptake by this non-hydrogenotrophic methanogen.

Objective (1) Determining the production of dissolved H_2 at the cathodic surface

The H_2 sensor, developed as reported by (6), was set up according to Figure 1. Argon gas was flushed into the headspace to maintain an anaerobic atmosphere. The working and counter electrodes were graphite rods and the reference electrode is a KCl saturated (Ag/AgCl) electrode.

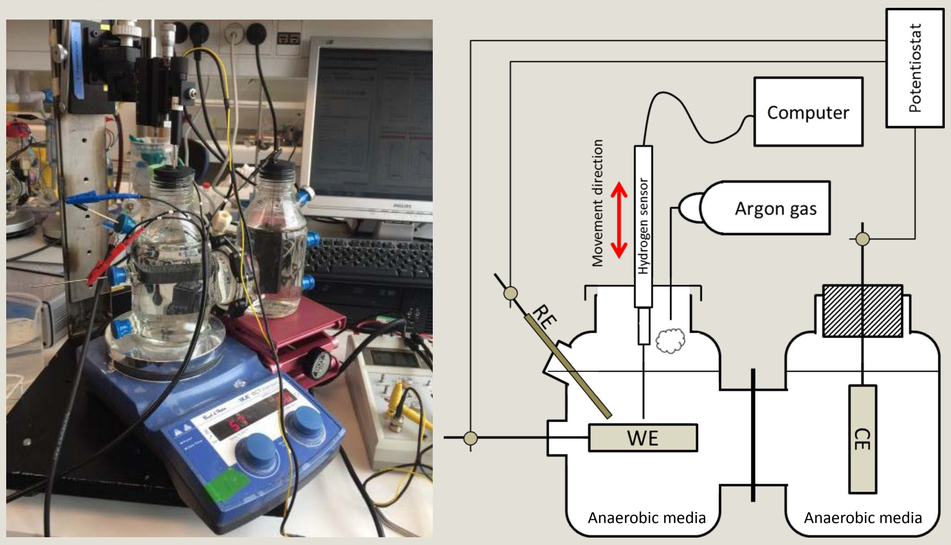

Figure 1 Schematic diagram of the H_2 sensor setup

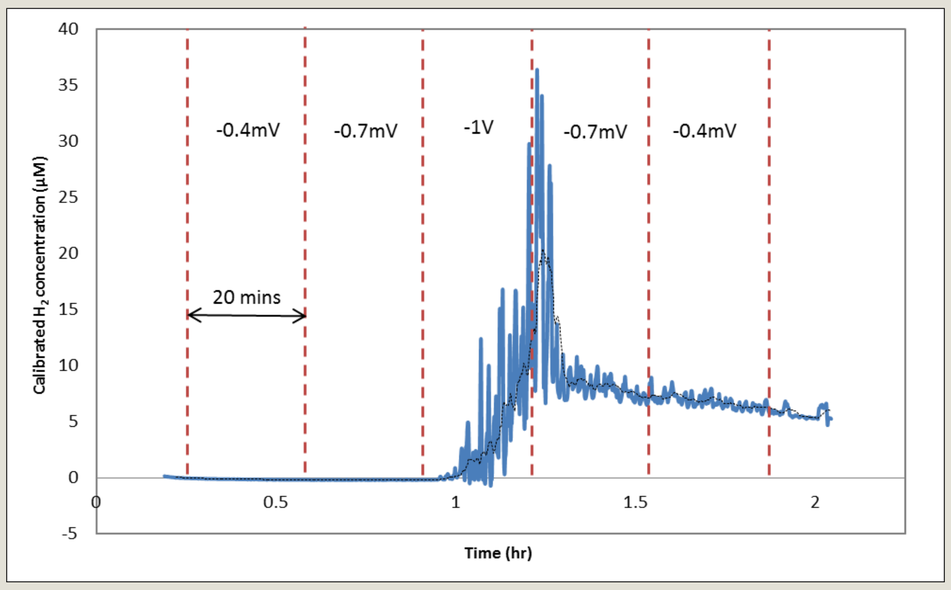

Figure 2 Instantaneous H_2 response as measured by the H_2 sensor at the cathodic surface at different poised potentials (vs. SHE) Blue solid line represents the calibrated H_2 concentration; Black dotted line represents the 20 pts moving average trendline

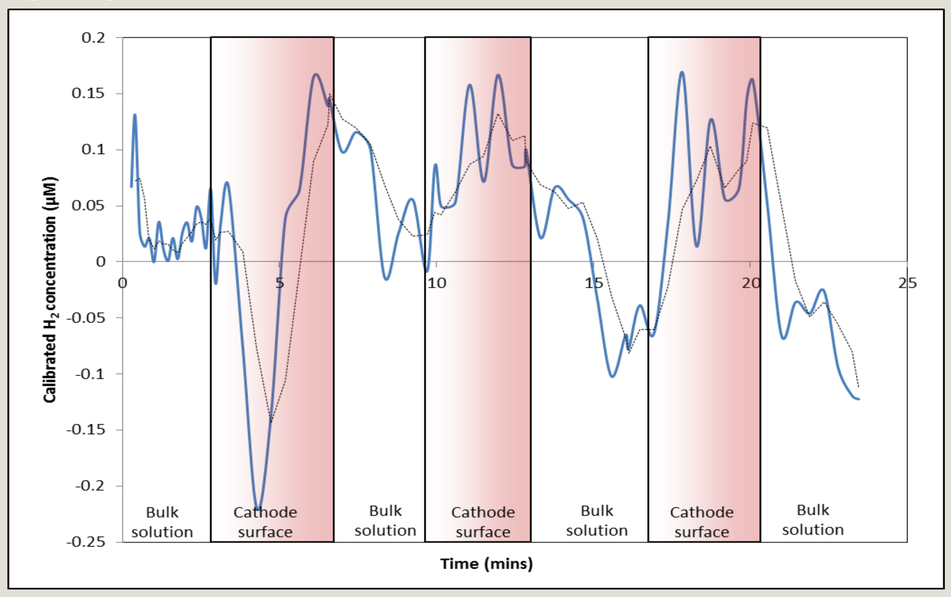

Figure 3 Spatial location of chemically produced hydrogen on the graphite cathodic surface with a poised potential of -400 mV (vs. SHE). Blue solid line represents the calibrated H_2 concentration; Black dotted line represents the 3 pts moving average trendline. The result is representative of 3 different reactors.

Discussion

Dissolved H_2 was measured using the highly sensitive sensor(sensitivity 7.7 pA/µM) at the cathodic surface. Preliminary data showed a slight change in concentration of H_2 (~0.2 µM) around the surface compared to the bulk solution at a poised potential of -400 mV (vs. SHE).However, due to the high background noise, more experiments are planned to validate this finding. Nonetheless, if indeed a concentration of 0.2 µM H_2 is electrochemically produced, it could enable the growth of hydrogenotrophs (Table 1) at the cathodic surface. Given that, we have focused our attention on studying electromethanogensis with strictly acetoclastic (eg. *M.horonobensis*) or poor H_2-utilizing methanogens (eg. *Methanosarcina barkeri*).

Organisms	Dissolved H_2 threshold at mesophilic conditions	Reference
Hydrogenotrophic methanogens; **- *Methanobacterium formicicum JF-1*** **- *Methanospirillum hungatei JF-1*** **- etc.**	6 – 70 nM	Lin et al. (2012) (8)
***Methanobacterium bryantii* M.o.H**	0.4 – 4 nM	Kardagali and Rittmann (2007) (9)
Methanosarcina barkeri	~296 – 376 nM	Kral et al. (1998) (10)

Objective (2) Investigating electromethanogenesis in a non-hydrogenotrophic methanogen

Methanosarcina horonobensis (MH) is a recently discovered acetoclastic methanogen that is unable to grow on H_2/CO_2. To examine if MH is capable of direct electron transfer we established co-cultures with *Geobacter metallireducens* (GM) and conductive granular activated carbon (GAC) with ethanol (Et) 10 mM as the sole substrate. A co-culture of *G. metallireducens* and *Methanosarcina barkeri* strain 227 (MB227) with GAC and ethanol was also set up for comparison. Previous studies with *Methanosarcina barkeri* strain 800 and GM did show that DIET occurred without conductive GAC (7).

A preliminary experiment for electromethanogenesis of *M.horonobensis* in an H-cell reactor poised at -400 mV (vs. SHE) was also carried out with a DC convertor. The media contained no added substrates or possible redox shuttles.

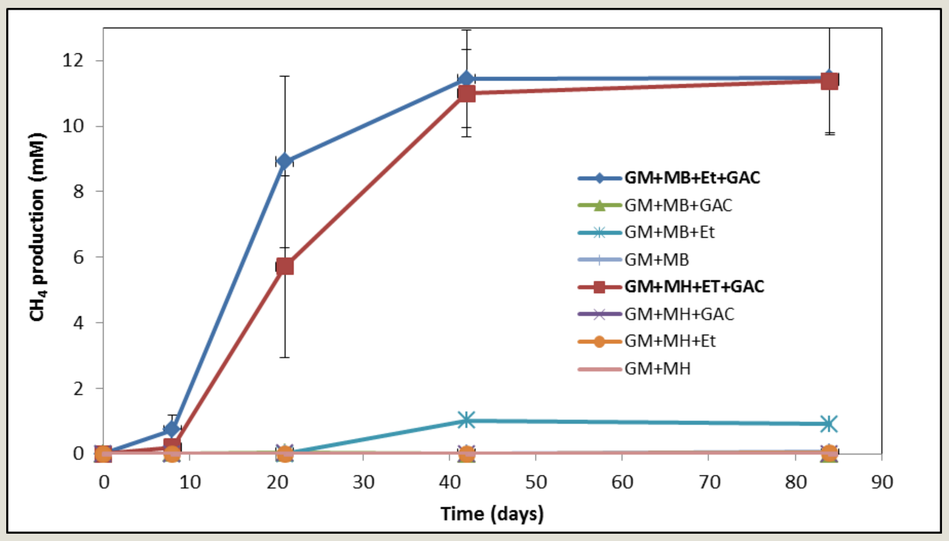

Figure 4 Methane production in co-cultures of *G.metallireducens* and *M.barkeri* or *M.horonobensis*. The error bars represent standard error of 3 replicates.

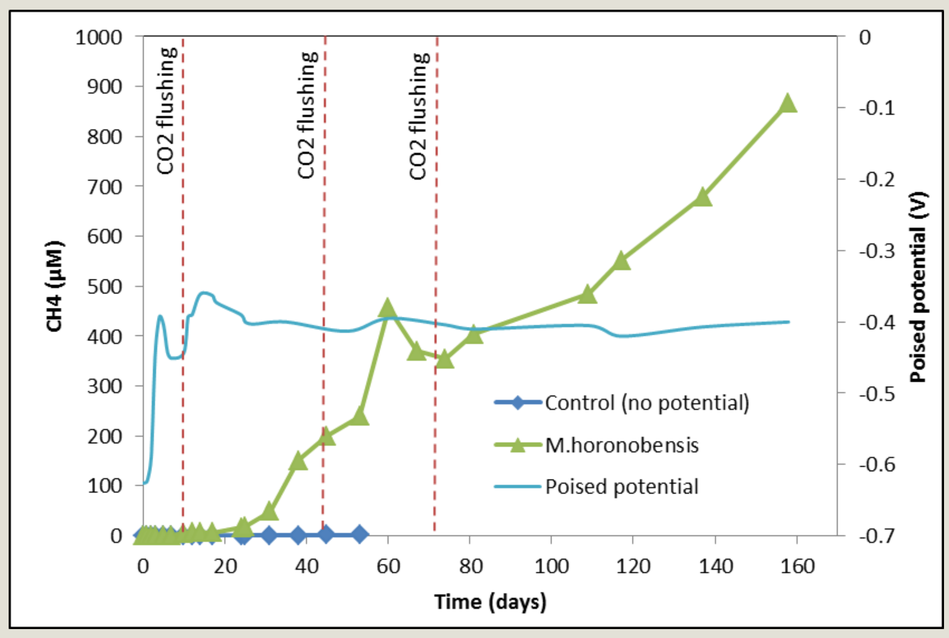

Figure 5 Methane production of *M.horonobensis* by electromethanogenesis poised at -400mV (Vs.SHE) with a DC convertor

Discussion

The co-culture of *M.horonobensis* and *G.metallireducens* showed that the methanogen has the ability to withdraw electrons deposited onto GAC by the electrogen. The rate of CH_4 production is similar to that of a *M.barkeri 227/G.metallireducens* co-culture, and surprisingly faster than described MB800/GM co-cultures with GAC (13.6 µmol/day vs. 5.384 µmol/day respectively) (7). More experimental data is needed to determine if the same mode of electron transfer (DIET) occurs in these new co-cultures.

M.horonobensis is able to carry out electromethanogenesis albeit at low levels, when using a DC source . We are now testing *M. horonobensis* using a potentiostat with a better controlled voltage and plan to determine if the mechanism is indeed DEET rather then enzymatic or shuttle-assisted.

Conclusion

Our preliminary studies has shown that a non-hydrogenotrophic methanogen could produce methane from electricity. At -400 mV (vs. SHE), H_2 could potentially be electrochemically produced at the cathodic surface. Both findings require further verification. We hypothesise that methanogens with the right affinity for H_2 could be favoured by the low- H_2 conditions at the cathode. Yet, whether the amount of H_2 produced at the cathode is significant for methanogens from anaerobic digesters still needs to be elucidated.

References

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