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## Enrichment strategy of chemolithotrophic biofilm for nitrogen fixation in MES

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### ABSTRACT

Nitrogen fixation (from  $N_2$  to  $NH_4$ ) is the first step in the transformation of atmospheric nitrogen into nitrogen that can be assimilated by living organisms. This reaction is catalysed by so-called diazotrophic microorganisms that reduce atmospheric nitrogen into ammonia to fuel their growth. Biomass production by  $N_2$  and  $CO_2$  fixation via electrostimulation in a microbial electrochemical system has been shown recently (Rago et al. 2019). We propose to investigate enrichment strategies allowing the selection of a cathodic biofilm capable of growing from the present gases and a mineral energy supply.

Two enrichment methods were tested. The first one focused on enriching nitrogen-fixing bacteria in C-organic supplied medium. Then a MES was used to enrich chemolithoautotrophic bacteria capable of using  $CO_2$  and electrons supplied at the cathode. The second strategy focused on the enrichment of chemolithoautotrophic bacteria before enriching nitrogen-fixing bacteria without using MES and with  $H_2$  as energy source.

We used dual-chamber reactors separated by AEM with a three electrodes set-up. The carbon felt working electrode was polarized at  $-0.7$  V vs SHE (pH 6,5-7) when OCV were used as control.

The rate of nitrogen fixation measured after 200 days in the MES without organic-C nor assimilable N was higher than in the second enrichment method ( $60.8 \pm 11.5 \mu\text{mol}_{C_2H_4}/L_{\text{gaz}}.d$  versus  $12.7 \pm 2.6 \mu\text{mol}_{C_2H_4}/L_{\text{gaz}}.d$ ). About 7 times more 16S rRNA copies/mL were observed in the medium of the MES than in the medium of the second method with  $H_2$ -supply. Enrichment led to a biofilm accumulation during 87 days in MES with  $N_2$  and  $CO_2$  as sole nitrogen and carbon sources respectively. Acetate was also measured up to 0.75 g/L after 15 days suggesting that bacteria are able to produce acetate with  $CO_2$  as carbon source in our first enrichment strategy. 16S rRNA sequencing showed the presence of *Hydrogenophilaceae*, *Chitinophagaceae* and *Rhodospirillaceae* (*Azospirillum*) in MES, known as nitrogen-fixing bacteria.

Finally, these results confirm that our first enrichment method had successfully enrich a microbial community able to fix  $N_2$  and  $CO_2$  in a cathodic biofilm.

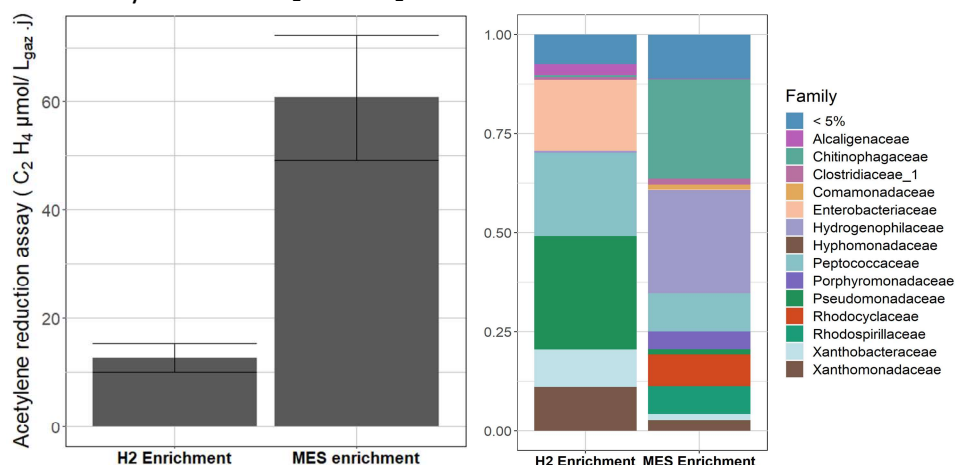


Fig 1. Comparison of Acetylene Reduction Assay (nitrogen fixation rate) between enriched biofilm in MES and  $H_2$  enriched community

Fig 2. Bacterial community of the two enrichments for chemolithoautotrophic nitrogen fixing bacteria

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### References:

Rago, Laura et al. 2019. « Bioelectrochemical Nitrogen Fixation (e-BNF): Electro-Stimulation of Enriched Biofilm Communities Drives Autotrophic Nitrogen and Carbon Fixation ». *Bioelectrochemistry* 125: 105-15.