Enrichment strategy of chemiolithotrophic biofilm for nitrogen fixation in MES

Axel Rous, Eric Trably, Elie Desmond-Le Quéméner, Nicolas Bernet

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INRAE, Univ Montpellier, LBE, 102 avenue des Etangs, 11100, Narbonne, France

Corresponding author email: axel.rous@inrae.fr

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 use 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 ± 11.5 $\mu mol_{C2H4}/L_{gaz}.d$ versus 12.7 ± 2.6 $\mu mol_{C2H4}/L_{gaz}.d$). 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.

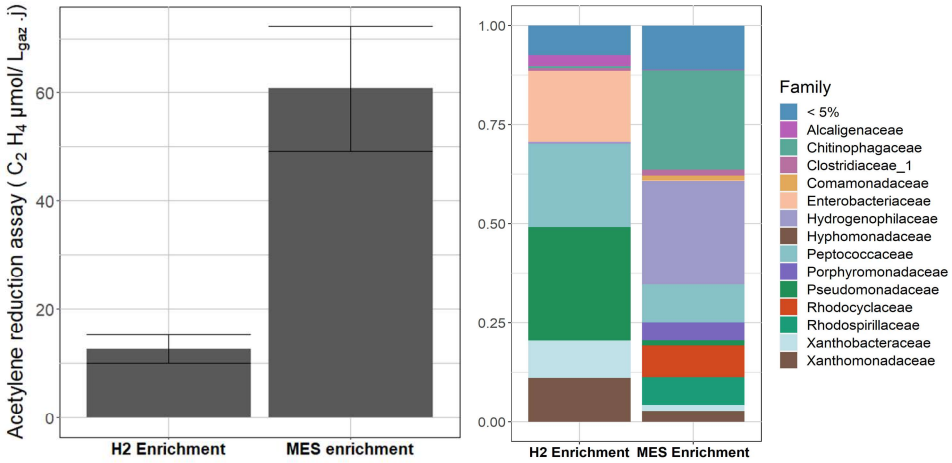

Fig 1. Comparison of Acetylene Reduction Assay (nitrogen fixation rate) between enriched biofilm in MES and H_2 enriched community

Fig 2. Bacterial community of the two enrichments for chemiolithoautotrophics 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.