**A new methodology for the selection of a methanogen-free acetogen inoculum from mixed sludge**

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**1.Introduction**

Mitigation of CO2 represents a major challenge to reduce emissions by 2030 as a global goal to limit global warming to an increase of 1.5 °C. The recycling of CO2 through its fixation into precious metals such as fuels and chemical compounds represents an interesting strategy in the industrial sector where concentrated CO2 flue gases are available such as the production of cement and steel or the treatment of wastewater. Many groups of microorganisms are able to biocatalize similar reactions through the Wood-Ljungdahl pathway (WLP) [1], where CO2 is fixed in acetate. Acetogens are ubiquitous and often share the same environments with methanogens, so to obtain an acetogenic inoculum from environmental samples it is necessary to inhibit methanogenesis [2]. This work proposes an innovative method for the inhibition of methanogenesis and the selection of acetogenesis based on the spore-forming nature of many acetogens. In fact, sporulation can be induced through multiple stresses, which simultaneously contribute to the inactivation of methanogenesis. the tests consisted in inducing sporulation, activating the biomass growth, and maintaining an active autotrophic community in hydrogenophilic condition growth.

**2. Methods**

*2.1**Sludge treatment and inoculum activation*

Figure 1 shows the sludge treatment and the preparation of the tests. Furthermore, part of the sludge was kept untreated to obtain the control suspension. The experimental se-up involved a first step in mixotrophic conditions, i.e. in the presence of organic substance and H2, and a second step in hydrogenophilic autotrophic conditions (only in the presence of H2 as electron donor), under hydrogenophilic autotrophic conditions, forcing the selected biomass to grow on H2 and CO2.

*2.2 Analytical methods*

The detection of H2, CO2, CH4 was carried out with gas chromatographic analysis of the headspace using a Dani Master GC equipped with a packed column and TCD detector set with the following conditions: injection volume 50 µL, carrier gas nitrogen, flow 12 mL/min, inlet temperature 120 °C, column temperature 70 °C and TCD detector at 150 °C. Organic carbon was measured by TOC (total carbon analyzer)-V CSN (Shimadzu) on filtered samples with cellulose acetate filters, diameter 25 mm, pore diameter 0.2 µm. The biomass was monitored through the spectrophotometric reading and correlated to the concentration expressed in VSS (volatile suspended solids, mg/L).

**3. Results and discussion**

*3.1* *Microbial growth in mixotrophic and autotrophic conditions*

**Figure 1.** Biomass profile over time in mixotrophic (A) and autotrophic (B) conditions. The tests are denominated T= thermal shock; TA=thermal and acid shock; A= acid shock.

The speed of biomass in mixotrophic conditions (A) highlights the activation of the biomass following the different treatments. The control test shows a higher growth rate, considering however that this growth is also attributed to methanogens (methane data not reported). growth rate under autotrophic conditions (B) decreased for all tests, as expected. The test treated with acid and thermal shock showed a growth rate close to the control test (12 mgVSS/Ld vs 15 mgVSS/Ld), considering that the methanogenesis in the first was inactive, while in the control 0.3 mmol/Ld was produced.

**4. Conclusions**

The treatments contributed to the inactivation of methanogenesis and to the activation of the acetogenic autotrophic community, previously activated in mixotrophic conditions and subsequently maintained in autotrophic conditions, where methane production was not observed.

**References**

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