Inoculum characterization: identification of acetate consumption routes

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HIGHLIGHTS

A mesophilic inoculum, likely to have syntrophic acetate oxidation bacteria (SAOB) and hydrogenotrophic methanogen (HM) activity due to operational conditions, was characterized by means of methanogenic activity tests, submitting it to different acetate and ammonia nitrogen (TAN) concentrations. Results showed a decreased HM activity and an increased acetoclastic activity, supporting the hypothesis that this inoculum presents SAOB-HM activity, which coexists with acetoclastic methanogens at low TAN concentrations.

Keywords
Syntrophic acetate oxidation bacteria, ammonium, hydrogenotrophic methanogens, acetoclastic methanogens

INTRODUCTION

Ammonia and acetic acid are considered important factors for methanogens metabolism. Concentrations higher than 3 g Ac/L and higher than 3 gTAN/L are reported to inhibit acetoclastic methanogens (AM) (Chen et al., 2008), but in lesser extend the hydrogenotrophic methanogens (HM) (Fotidis et al., 2013). Moreover, some HM are able to cooperate with the syntrophic acetate oxidation bacteria (SAOB) which have been reported to develop when ammonium and/or acetate concentrations reach inhibitory values for AM (Schnürer et al., 1999; Hao et al., 2013). The aim of this work is to study the coexistence of acetate consumers in a mesophilic anaerobic inoculum, using usual methanogenic activity tests.

MATERIALS AND METHODS

The inoculum was collected in a mesophilic digester (4.2 gN-TAN/L, 80.9 g COD/L, 53.5 gVSS/L, pH 8.3, 0.1 gAc/L), usually fed with protein-rich wastes at 65 days HRT. Inoculum (7.5 gVSS/L) was added to glass vials (0.7 L working volume) containing different concentrations of acetate and TAN. Identical acetate and H_2 amounts were added in three pulses, while TAN was only added at the start. Two sets of vials (duplicate at 37ºC) were studied: vials 1-4, containing 0.01-3.2 gAc/L, were used to study the effect of increasing COD content; vials 5-8, containing 0.7-2.4 gTAN/L and equal acetic content (3.2 gAc/L) were used to study the AM inhibition. A phosphate buffer solution was added to keep a neutral pH. Specific consumption or production rates (gCOD/gVSS·d) were determined within the first days after each pulse: acetate or hydrogen consumption rates (r_{Ac} and r_{H2}) and methane production rate (r_{CH4}).

RESULTS

Although the inoculum consumed the added H_2 in the first three days after each pulse and kept it on negligible values afterwards, the H_2 consumption rate slightly decreased throughout pulses. Acetate concentration decreased with different rate in the case of vials 1-4, as expected since r_{Ac} is acetate-concentration depending (Figure 1), and with the same rate in the case of vials 5-8. This rate consumption showed a clear drop regarding TAN concentration: on 1^{rst} pulse, r_{Ac} decreased as TAN increased, with an upper value at 1.3gTAN/L and a lower value at 2.5gTAN/L (Figure 1). On the

2nd pulse, r_{Ac} presented a very disperse distribution: in average it increased amenably and seemed to be less sensitive to ammonia than in 1rst pulse. The AM activity increase and the simultaneous HM activity decrease of the inoculum could be explained due to its source: the HM activity in the digester is favoured due to operational conditions over the acetoclastic activity that is reported to be more sensitive at high TAN concentrations. Angelidaki and Ahring (1993) found an inhibition effect on AM within a 1.5-2.5 g TAN/L range, which matches with the observed AM activation at TAN content below 2.5 g/L.

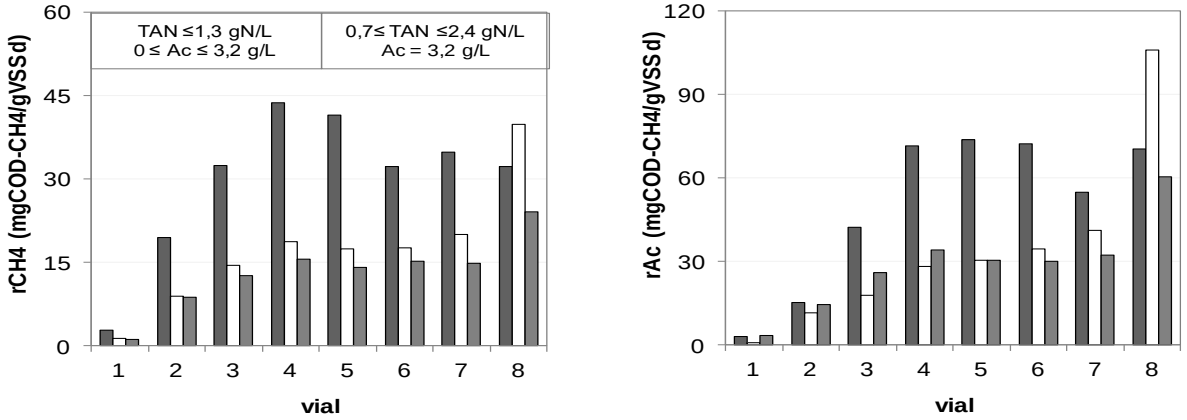

Figure 1. Specific rates: (left) methane production rate (r_{CH4}); (right) acetate consumption rate (r_{Ac}). Colours: dark grey – pulse 1; white – pulse 2; light grey – pulse 3

The cumulative methane production reached the expected values even there was small decrement from 1st to 2nd pulse due to the consumption of residual COD of the inoculum. Likewise r_{H2}, the r_{CH4}diminished in successive pulses (Figure 1): although an overall increment was observed in 2nd and 3rd pulses, this rate represented 1/3 of the 1rst pulse r_{CH4}. Besides this, the COD balance showed that the COD recovered as methane tended to diminish as TAN increased, with the exception of vials 8 after 2nd pulse, where an apparent COD unbalance was found since methane production did not fit the measured consumed acetate. Further analysis regarding microbial population community of these vials pointed out other acetate consumers different than methanogens.

CONCLUSIONS

Observed changes on the methanogenic activity of the studied inoculum were consistent with its origin, a digester characterized by high hydrogenotrophic activity, low acetoclastic activity and high TAN concentration. After submitting the inoculum to different and relatively low TAN concentrations, a clear shift of the methanogenic activity was observed: the HM activity decreased almost three times at the end of the assay while the AM activity showed a slight increase. Results support the hypothesis that the inoculum evolved towards a community where SAOB-HM pathway remain active and coexist with the acetoclastic methanogenic route.

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