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How much microbial genetic expression profile, depending ammonia stress, of anaerobic digesters change?

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Abstract
Biomass from two full-scale anaerobic digester treating different wastes regarding to nitrogen concentrations and operating under different hydraulic retention time was characterized by methanogenic batch activity assays at different ammonia concentrations. Two several different patterns regarding activity and microbial parameters was demonstrated depending on operational conditions and methanogenic pathway. Furthermore, syntrophic acetate oxidation (SAO) process could be a possible alternatively pathway to obtain more methane enrich biogas.

Keywords
Biogas; ammonia; syntrophic-acetate-oxidation; hydraulic retention time

INTRODUCTION

Biogas production, through the biologically mediated process anaerobic digestion (AD), from industrial and agricultural wastes is a particularly attractive way to generate renewable and versatile energy, as methane, that could be available for electricity and/or heat generation. However, most of energy potential wastes that food processing industries provide, such as animal manures and slaughterhouse by-products, are nitrogen-rich materials which prompted the generation of ammonia under anaerobic conditions. High concentration of ammonia may indeed lead to unstable performance and operational failure of full-scale anaerobic digesters, due to inhibition of methanogens, leading to is a suboptimal biogas production (Hansen et al., 1998). Methane is produced through direct acetic acid cleavage by acetoclastic methanogens (AM) or through a tandem reaction between syntrophic acetate oxidation bacteria (SAOB), which oxidise acetate to CO_2 and H_2/ formate, and hydrogenotrophic methanogens (HM) that consume H_2 with subsequent CH_4 production (Schnürer, et al 2008). As Fotidis et al., (2014) described, under ammonia stress, AM are usually more susceptible to be inhibited by ammonium than HM. De Baere et al (1984) cited that 1700-1800mg/l of TAN is completely inhibitory concentration to unacclimated inoculum, although with acclimation, inhibitory TAN levels could increase up to 5000mg/l. For these reason three different concentrations above and under inhibitory concentrations described will be test in the ongoing study.

Some strategies to overcome or mitigate ammonia inhibition have been used (ie. dilution with water, low working temperature, increase C/N ratio, etc.) in full-scale process, but in some cases these are expensive and/or time-consuming (Cheng, et al 2008). Furthermore, little is known about the advantages in terms of methane production, degradation efficiency and biogas purity front the HM and SAOB syntrophic process, being the enrichment of microorganisms involved in the HM-SAOB consortia an innovative strategy to avoid ammonia stress. The aim of this work is to present an integral characterization procedure for anaerobic inocula by means of an interdisciplinary research, which combined batch methanogenic activity assays with quantification of microbial population through the expression level of relevant genes by reverse transcription qPCR (RT-qPCR). Secondary, the presence of HM-SAOB consortia in industrial digesters detected through this procedure. For that purpose, inocula from two different full-scale digesters subjected to different temperature, hydraulic retention time (HRT) and total ammonia nitrogen (TAN) concentration, were collected and exposed to increasing TAN levels.

MATERIALS AND METHODS

Two inocula (I1, I2) were characterised. The mesophilic inoculum I1 was collected from a 1,500 m^3 full-scale stirred anaerobic digester with low-solids concentration (Lleida, Spain), operated at a HRT of 50 days and treating pig manure and other protein-rich wastes (4.1 gTAN L^{-1}). The thermophilic inoculum I2 was collected from a 2,000 m^3 full-scale vertical digester with high-solids concentration (Barcelona, Spain), operated at a HRT of 17 days and treating organic fraction of municipal waste (2.1 gTAN L^{-1}).

Specific methanogenic activity (SMA) assays at 35º and 55ºC (12.7 and 25.8 gVSS L^{-1} for the mesophilic and thermophilic inocula, respectively) were carried out in triplicate with three TAN concentrations (1, 3, 6 gN-TAN L^{-1}). All vials were flushed with N_2 gas in the starting moment, and therefore acetic acid (2.5-3.0 g L^{-1}) was added. Controls or vials without acetic were also performed at each temperature. The evolution of the headspace components (CH_4, CO_2), volatile fatty acids (VFA) and TAN of the liquid media were monitored along the assay. The activity was expressed as a specific and net methane production rate ($mg_{COD\text{-}CH4}$ g_{VSS}^{-1} d^{-1}). Biomass samples for RNA extraction were collected from each vial at exponential phase of each pulse of acetate and kept at -80^0C. Expression levels of relevant genes (*16S rRNA* and *mcrA)* was quantified by reverse transcription qPCR (RT-qPCR) as described (Sotres et al., 2014).

RESULTS

Combined quantification of activity through batch assays and gene expression was performed. Methane yield and SMA of both inocula I1 and I2 were determined; Figure 1 shows the average values of triplicates. At low TAN level where no inhibition was expected, both inocula yielded similar values but with remarkable different SMAs (Fig. 1). Regarding gene expression (Figure 2), eubacterial RNA transcripts were similar, while archaeal RNA of I2 was lower than in I1. Based on results, it was found that activity measured by RT-qPCR and SMA from batch assays were not correlated. High number of *mcrA* copy transcripts of I1 at 1 N-TAN L^{-1} level was determined, although a low SMA were attained. Opposite, almost a 2 times greater SMA of inoculum I2 than I1 was obtained although a lower number of *mcrA* copy transcripts in I2 (Figure 2). In addition, the methane richness, expressed as the ratio between methane and the sum of methane and carbon dioxide, of the produced biogas was monitored; inoculum I1 reached higher CH_4 values of 92% than inoculum I2, with 86%.

Ammonia concentration above 1 gN-TAN L^{-1} suppose a large disturbance in relation to efficiency and methanogenic activity; so the relative evolution of each inocula at 3 and 6 g N-TAN L^{-1} was compared to parameters obtained at 1 g N-TAN L^{-1}. The inoculum I1 showed a minor change in terms of yield and activity regarding increasing TAN concentration, while in contrast I2 presented a sharp decrement in both parameters when TAN concentration increased. The SMA of I2 was directly correlated to TAN concentration: a decreasing trend of SMA regarding increasing TAN levels was shown (Fig.1 right). A similar behaviour was observed in terms of yield in the case of I2 vials; longer VFA than acetic were found in the liquid media at the end of this assay (data not

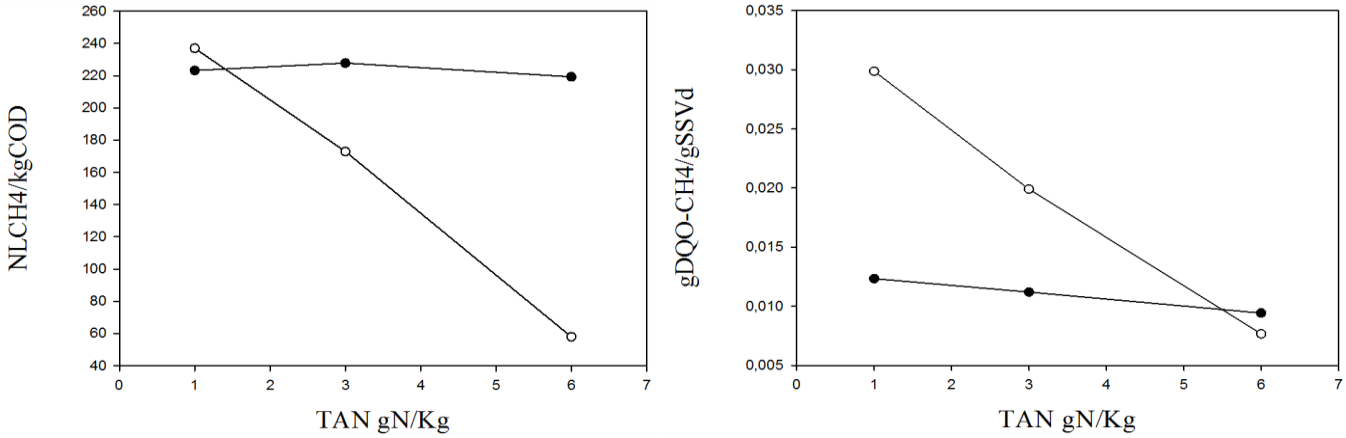

Figure 1 Methane yield (left) and methanogenic activity (right) of the mesophilic and thermophilic inocula. Note: solid symbols represent mesophilic inoculum I1, operated at high HRT; empty symbols represent thermophilic inoculum I2, operated at low HRT

shown). Regarding the methane richness, it was obtained a greater methane quantity in I1 vials, with CH_4 values of 86% and 83% when the ammonia concentration was 3 and 6 g N-TAN L^{-1}, respectively. In contrast, CH_4 values of 82% and 79% were obtained with inoculum I2 for 3 and 6 g N-TAN L^{-1}, respectively. These results are in accordance with stoichiometry of both reactions because HM metabolize CO_2 to produce methane:

AMA $CH_3COOH \rightarrow CH_4 + CO_2$

SAO-HM $CH_3COOH + H_2O \rightarrow 2CO_2 + 4H_2 \rightarrow CH_4 + CO_2$

Concerning the analysis of the microbial community, the partial inhibition of activity observed at the highest assayed TAN value was consistent with gene expression profiles (Figure 2). Eubacterial RNA transcripts from *16S rRNA* genes didn't change regarding TAN level, while methanogenic archaea (*mcrA* genes) varied depending upon inocula. Maximum expression of *mcrA* was obtained at lower TAN concentration in both inocula, while it clearly decreased in inoculum I2 as TAN increased. Furthermore, the ratio of *mcrA* copy transcripts/*16S rRNA* copy transcripts normalized to *16S rRNA* was done, normalizing to *16S rRNA* (Fig. 2), showing that inoculum I1 profile was stable when ammonia concentration increased, however this ratio decreased severely in the inoculum I2 at 3 and 6 g N-TAN L^{-1}.

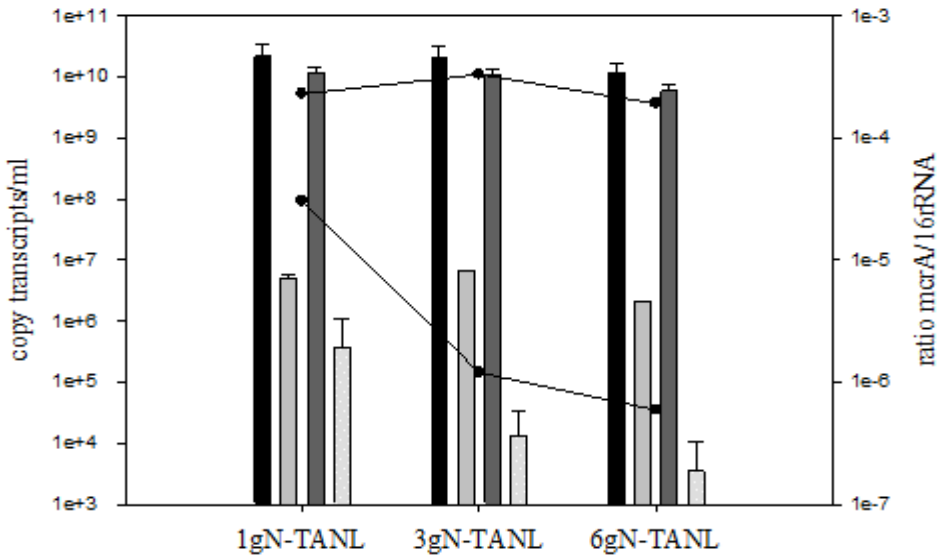

Figure 2 Quantification of microbial populations. Note: first and second bars denote 16S rRNA and mcrA of inoculum I1; third and fourth bars denote 16S rRNA and mcrA of I2; solid and empty circles represent the cDNA mcrA/16S rRNA ratio, normalized to 16S rRNA copies, of inocula I1 and I2, respectively.

In the case of I2, the microbial population were operated at low HRT of 17 days which might not favoured the growth of SAOB and SAOA populations, which have high duplication time (Schnürer et al 1999). In this case, AM pathway might be the control methanogenesis route since a clear decrement on SMA and gene expression was found in comparison with non-inhibited state with 1 g N-TAN L^{-1}. Regarding the constant SMA and gene expression although increased TAN levels of I1, it can be hypothesized that SMA of this inoculum could be considered as an "apparent" activity of acetate consumers moreover than HM alone. Regarding the source of inoculum I1, it was previously operated at such high HRT of 50 days that SAOB growth could be attained. Based on these results, it can be concluded that I1 had more stability in terms of higher activity rates and higher efficiency toward ammonia stress.

CONCLUSIONS

The integrated characterization of biomass specific activity rates, efficiency, genetic expression profiles and biogas richness provided evidence on the occurrence of HM-SAOB activity in one of the studied inocula. Furthermore, the results obtained from methane richness (92% and 87% for inocula I1 and I2, respectively) suggested that tandem reactions involved in SAOB-HM process could improve the biogas to energy yield. In addition, the genetic expression profile suggest another possible pattern to differentiate AM to SAOB-HM process, since results showed that cDNA *mcrA*/*16S* ratio was kept constant when ammonia concentration increased.

All results indicated that different conditions of acclimatization influence strongly on most important parameters involved on anaerobic reactor operation.

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