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Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters

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ABSTRACT

High activity levels and balanced anaerobic microbial communities are necessary to attain proper anaerobic digestion performance. Therefore, this work was focused on the kinetic performance and the microbial community structure of six full-scale anaerobic digesters and one lab-scale co-digester. Hydrolytic (0.6–3.5 g COD g^{-1} VSS d^{-1}) and methanogenic (0.01–0.84 g COD g^{-1} VSS d^{-1}) activities depended on the type of biomass, whereas no significant differences were observed among the acidogenic activities (1.5–2.2 g COD g^{-1} VSS d^{-1}). In most cases, the higher the hydrolytic and the methanogenic activity, the higher the *Bacteroidetes* and *Archaea* percentages, respectively, in the biomasses. Hydrogenotrophic methanogenic activity was always higher than acetoclastic methanogenic activity, and the highest values were achieved in those biomasses with lower percentages of *Methanosaeta*. In sum, the combination of molecular tools with activity tests seems to be essential for a better characterization of anaerobic biomasses.

1. Introduction

Anaerobic biodegradability or biomethanation potential tests are commonly used to evaluate the susceptibility of an effluent to be treated anaerobically. The inoculum for these tests should be "fresh" from any type of anaerobic reactor (sludge reactors, manure-based biogas reactors or sludge bed reactors) in order to ensure that different substrates would not face any limitations (Angelidaki et al. 2009). The quality of the inoculum is usually previously checked by performing activity tests, whose results will provide information about the experimental time or the amount of inoculum required. In addition, the selection of the waste-to-inoculum ratio is also crucial when evaluating the anaerobic biodegradability of solid wastes (Lopes et al. 2004), in order to obtain a better control of the process, and thus avoiding overload or process failure.

On the other hand, start-up is considered the most critical step in the operation of anaerobic digesters. Since methanogenesis is usually considered the rate-limiting step, the start-up time of anaerobic digesters often ranges from 3 to 5 months depending on the characteristics of the substrate treated (Lettinga 1995), as a result of the low growth rate of methanogenic *Archaea*, about 0.03 kg of volatile solids (VS) produced per kg of chemical oxygen demand (COD) removed (van Haandel and Lettinga 1994). Selecting a highly active anaerobic inoculum will reduce significantly the start-up time or the amount of inoculum required, and consequently, will increase the economical competitiveness of anaerobic processes (Escudié et al. 2011).

In the past, methanogenic activity was the main selection criteria to choose a good inoculum. However, when dealing with solid substrates, methanogenesis might not be the limiting step. Moreover, for a good-performing anaerobic process, an adequate balance between all the microbial populations involved should be pursued as well (McMahon et al. 2004). Additionally, the presence of active microbial populations, known to play an important role during anaerobic digestion, could also help to make the final decision. Such studies have been difficult in the past because of the recognized limitations of traditional culture-based methods, but nowadays, the application of molecular-based methods has facilitated studies about microbial architecture (Amann et al. 1995).

The purpose of this study was to evaluate the microbial activities (hydrolytic, acidogenic and methanogenic) and the microbial community structure characterized by denaturing gradient gel electrophoresis (DGGE) and fluorescence in situ hybridization (FISH) in six full-scale anaerobic digesters and one lab-scale co-digester, with the aim of establishing a relationship between them. The overall hypothesis is that a better understanding, not only of the microbial activities, but also of the active microbial populations

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present in anaerobic biomass can help to better choose the seed sludge source to inoculate anaerobic reactors, and consequently, avoid a poor start-up with a prolonged period of acclimation.

2. Materials and methods

2.1. Full-scale anaerobic digesters

Six full-scale anaerobic reactors treating a variety of residues were sampled: a sludge digester from a sewage treatment plant (STP); anaerobic digesters from a brewery, a dairy, a sugar industry and a yeast industry; and a co-digester treating dairy and fish waste (DF). Additionally, a lab-scale co-digester treating slaughterhouse waste with pig manure and glycerine (SPG_{lab}) was also included in the study. The operational parameters of the reactors during the sampling period are shown in Table 1. All digesters were continuously stirred tank reactors, except the brewery digester that was an upflow sludge bed reactor, and they were working in mesophilic range. The organic loading rate (OLR) varied from 1 to 8.3 kg COD m^{-3} d^{-1} and the hydraulic retention time (HRT) ranged from 0.4 to 30 d.

2.2. Hydrolytic, acidogenic and methanogenic activities

All the activity tests were performed in triplicate and the protocol described by Soto et al. (1993) was taken as reference.

2.2.1. Specific hydrolytic activity (SHA)

The assays were performed in 122 mL volume serum flasks (100 mL of working volume) and with a biomass concentration of 1.5 g volatile suspended solids (VSS) L^{-1}. A blank without inoculum was included in order to measure the abiotic disappearance of the substrates. Two substrates (starch and glycogen) were used with a concentration of 1.5 g COD L^{-1}, in order to maintain an inoculum-to-substrate ratio of 1:1. Samples of the supernatant (1.5–3 mL) were taken every 2–3 h to determine the remaining starch/glycogen concentrations in the flasks. The evolution of the concentration of substrate (expressed in g COD L^{-1}) versus time was plotted and the SHA was calculated as the ratio between the maximum slope of disappearance of substrate (g COD L^{-1} d^{-1}) and the concentration of biomass used (g VSS L^{-1}).

2.2.2. Specific acidogenic activity (SAA)

A similar procedure as described for the SHA was used, but with glucose as substrate.

2.2.3. Specific methanogenic activities

The specific methanogenic activity (SMA), the specific acetoclastic methanogenic activity (SAMA) and the specific hydrogenotrophic methanogenic activity (SHMA) were determined using a mixture of volatile fatty acids (1.5 g COD L^{-1}, 50% acetic acid, 25% propionic acid and 25% butyric acid, COD basis), acetate (1.5 g COD L^{-1}) and H_2/CO_2 (80:20, v/v), respectively, as substrates. A blank with inoculum, but without substrate, was also included. Methane production over time was monitored and the methanogenic activity was calculated as the ratio between the maximum slope of the cumulative methane produced over time (g COD L^{-1} d^{-1}) and the concentration of biomass used (g VSS L^{-1}).

2.3. Microbiological analysis

2.3.1. DNA extraction

2 mL aliquots of well-homogenized biomass were stored at −20 °C before analysis and total genomic DNA was extracted using the phenol–chloroform protocol (Alonso-Gutierrez et al. 2009). DNA content was measured with Nanodrop and the extraction was considered satisfactory if this content was above 100 ng μL^{-1}.

2.3.2. Denaturing gradient gel electrophoresis (DGGE) and sequencing

Genomic DNA was subjected to DGGE analysis as previously described (Alonso-Gutierrez et al. 2009). The 16S rRNA gene hypervariable regions of *Bacteria* and *Archaea* were amplified by PCR using primers U968-f and L1401-r for *Bacteria* and primers A109(T)-f and 515-r for *Archaea* (Sousa et al. 2007). Primers U968-f and 515-r included a GC clamp at the 5′ end. PCR results (positive amplification) were verified in an agar gel. DGGE analysis were performed in 1× TAE buffer using an INGENY PhorU system (Ingeny, Goes, The Netherlands) at 100 V and 60 °C for 17 h. DGGE gels were stained with 1× TAE buffer containing SybrGold (Molecular Probes, Inc., Eugene, OR, USA). The urea/formamide gradient was 40–80% for *Bacteria* and 30–70% for *Archaea*.

Predominant DGGE bands were excised with a sterile razor blade, suspended in 50 μL sterilized milliQ water, stored at 4 °C overnight, re-amplified by PCR using the same primers without the GC clamp and sequenced. Sequencing was accomplished using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (version 3.1) and an ABI PRISM 3700 automated sequencer (PE Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Homologues search was conducted with the BLAST server of the NCBI using a BLAST algorithm (http://www.ncbi.nlm.gov.library.vu.edu.au/BLAST/).

2.3.3. Fluorescent in situ hybridization (FISH)

Active microbial populations were identified by the FISH technique. Fresh biomass samples were disrupted and fixed with 4% paraformaldehyde according to the procedure described by Amann et al. (1995). Hybridization was performed at 46 °C for 90 min adjusting the formamide concentrations for each probe. The probes used were: $Eub338_{mix}$ (*Bacteria*), Synm700 (*Syntrophomonadaceae*), CFB562 (*Bacteroidetes*), CFX1223 (*Chloroflexi*), ALF1b (*Alphaproteobacteria*), BET42a (*Betaproteobacteria*), GAM42a (*Gammaproteobacteria*), DELTA495a (*Deltaproteobacteria*), Arc915 (*Archaea*), MB1174 (*Methanobacteriales*), Ms821 (*Methanosarcina*), Mx825 (*Methanosaeta*), Arc864 (Arc1) and Eury496 (*Methanomicrobiales*). All the details of each probe (formamide percentage, sequence and target organism) can be found in the probeBase database (http://www.microbial-ecology.net/probebase/). All probes were 5′ labeled with the fluorochromes FITC and Cy3. Fluorescence signals were recorded with an acquisition system (Coolsnap, Roper Sicientific Photometrics) coupled to an Axioskop 2 epifluorescence microscope (Zeiss, Germany). DAIME program (Daims et al. 2006) was used to make the semi-quantitative counting with at least six photos taken per 20 μL of fixed sample (10^8–10^9 cells mL^{-1}).

2.4. Analytical methods

The physico-chemical characterization of the different anaerobic biomasses was performed according to Standard Methods (APHA 1998). The concentration of starch was calculated as the difference between the total sugars (Dubois et al. 1956) and the reducing sugars (DNS method) concentrations. Glucose concentration was determined using a commercial enzyme kit (GOD-PAP/Trinder; Spinreact). Glycogen was determined with a method based on the formation of the iodine-glycogen complex (San Pedro et al. 1994). Biogas production was monitored using the pressure transducer technique (Colleran et al. 1992) and biogas composition was analyzed by gas chromatography (HP, 5890 Series II).