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Outlining microbial community dynamics during temperature drop and subsequent recovery period in anaerobic co-digestion systems

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ABSTRACT

To improve the stability of anaerobic reactors, more knowledge is required about how the different communities react against operating perturbations and which specific ones respond better. The objective of this work was to monitor the changes in microbial community structure of an anaerobic digester during a temperature drop by applying different complementary molecular techniques. Temperature decrease led to an increase of *Bacteroidales* order, *Porphyromonadaceae* family and *Bacteroides* genus and a decrease in *Syntrophomonas* and *Clostridium* genera. Once the temperature was restored, the reactor recovered the steady state performance without requiring any modification in operational conditions or in the microbiome. During the recovery period, *Sedimentibacter* genus and *Porphyromonadaceae* family played an important role in the degradation of the accumulated volatile fatty acids. The hydrogenotrophic methanogens appeared to be the keystone archaeal population at low temperatures as well as in the recovery period. This study stands out that the understanding of microbial community dynamics during temperature drop could be utilized to develop strategies for the mitigation of temperature change consequences and speed up the recovery of stable reactor performance.

1. Introduction

Anaerobic digesters are often exposed to operational and/or environmental perturbations, such as temperature drops, organic overloadings, or the entry of toxic compounds, leading to instability due to a stressed microbial community (Kleyböcker et al., 2012). Reactor microbiome responds differently to distinct disturbances, resulting in an imbalance in the trophic network (Leitão et al., 2006). This usually causes an accumulation of intermediate products, such as volatile fatty acids (VFAs), which may in turn inhibit microbial numbers, eventually reducing the process efficiency. Allison and Martiny (2008) have indicated the necessity to understand the relationship between the microbiome and their function in the anaerobic process, especially in deteriorated systems in order to identify those populations that better respond to each individual operational stressor with the ultimate goal of improving the stability of anaerobic reactors and the control based on microbial management.

One common perturbation occurring in anaerobic reactors is temperature variations (Poh and Chong, 2009). Many studies have investigated microbiomes in anaerobic reactors working at different temperatures and they point out that community profiles varied in each temperature range. Lee et al. (2012) detected a large presence of *Firmicutes* in thermophilic range compared to mesophilic one, and the opposite trend for *Bacteroidetes* phylum. Leven et al. (2007) showed that *Chloroflexi* and *Bacteroidetes* represented the major phyla at mesophilic temperatures, whilst the phylum *Thermotogae* was the dominant group in the thermophilic range. In relation to the archaeal community, *Methanomicrobiales* dominated at 15 °C, and *Methanosaeta* and *Methanomicrobiales* were the most abundant at 37 °C (Bialek et al., 2012). Summarizing, these studies confirm the temperature as a parameter affecting the structure of the microbial communities, even more than the increase in the organic loading rate (OLR) (Guo et al., 2014), but they lack transient membership of the microbial community during the days of the temperature perturbation. Moreover, to the best of our knowledge, there have been no studies published, which monitored the microbial community dynamics during the recovery period after restoration of temperature. All this understanding could be utilized to develop strategies for the mitigation of temperature change consequences and speed up the recovery of stable reactor performance based on microbial knowledge.

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Table 1
Physico-chemical characteristics of pig manure (PM), molasses residues (MR) and fish waste (FW).

	PM[a]	MR	FW
TS ($g\,kg^{-1}$)	50 ± 15	835	304
VS ($g\,kg^{-1}$)	40 ± 10	707	282
TKN-N ($g\,N\,kg^{-1}$)	3.1 ± 1.0	57	19
NH_4^+-N ($g\,N\,kg^{-1}$)	2.8 ± 0.7	15	0.7
COD_{total} ($g\,O_2\,kg^{-1}$)	62 ± 20	723	567
TA ($g\,CaCO_3\,kg^{-1}$)	10.5 ± 3.0	N.D.	N.D.
PA ($g\,CaCO_3\,kg^{-1}$)	4.5 ± 2.0	N.D.	N.D.
Lipids ($g\,kg^{-1}$)	N.D.	N.D.	35

TS: total solids; VS: volatile solids; TKN-N: total Kjeldahl nitrogen; NH_4^+-N: ammonium; COD_{total}: total chemical oxygen demand; TA: total alkalinity; PA: partial alkalinity; N.D: not detected.

[a] Standard deviations are only shown for pig manure since different batches of this substrate were necessary during the whole experiment (over 400 days).

Therefore, the objective of this work was to monitor the changes in microbial community structure of an anaerobic reactor not only during a temperature drop from mesophilic (37 °C) to psychrophilic (17 °C) range but also during the recovery period after restoring temperature to 37 °C. The experimental design consisted of two types of perturbation (gradual and abrupt) and three complementary molecular techniques were applied to follow microbial community dynamics: Denaturing Gradient Electrophoresis Gel (DGGE), Fluorescence In Situ Hybridization (FISH) and a 16s rRNA characterization with Illumina MiSeq platform sequencing.

2. Materials and methods

2.1. Substrates and inoculum

Pig manure (PM) was obtained from a fattening hog farm (3000 heads) located in Santiago de Compostela (Spain). Fish waste (FW) was collected in a canning industry located on the Galician coast (Spain) and consisted of processing remains of albacore. Beet molasses residues (MR) were obtained from a sugar processing company in A Coruña (Spain) and consisted of by-products of the sugar-extraction process. Fish waste was grounded prior to use and the substrates were stored at 4 °C. All residues were characterized (Table 1) in terms of total and volatile solids content, total Kjeldahl nitrogen and ammonium concentration, total chemical oxygen demand (COD), total and partial alkalinity, and lipid content.

A mixture (50:50, v/v) of two anaerobic sludges, one from a sewage sludge anaerobic digester and the second from a brewery wastewater anaerobic reactor, was used as inoculum. The initial in-reactor inoculum concentration was 15 g of volatile suspended solids (VSS) per liter.

2.2. Anaerobic digester operation

2.2.1. Experimental setup

One continuously stirred tank reactor (160 rpm, Heidolph RZR 2041), with a working volume of approximately 10 L, was operated in two consecutive experimental runs (Experiments 1 and 2). The reactor was fed semi-continuously (once a day draw-off and feeding) with a mixture of PM, FW, and MR (60–20–20, COD basis) during both experiments. This mixture was prepared every week, diluted with tap water according to the applied OLR, and stored at 4 °C. Temperature, pH, stirring speed, biogas production (Ritter milligascounters, Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany) and biogas composition (AwiFLEX model from AWITE Bioenergie GmbH) were monitored on-line. Samples of reactor mixed liquor were taken twice a week for VFAs, total COD, total suspended solids (TSS), VSS, alkalinity and ammonium determinations. Biomass samples were taken weekly for molecular analysis, except during the perturbation period, when a more frequent sampling was conducted (every 2–3 days).

2.2.2. Operational strategy

Experiment 1 was divided in three periods: start-up (0–30 days), gradual increase of OLR from 0.5 to 2 g COD L^{-1} d^{-1} and steady state performance at the latter OLR (31–150 days), and the perturbation period (151–195). The same periods were applied in Experiment 2, but the perturbation period lasted from day 151 to 161 and a fourth period, which we refer to as the recovery period (162–212 days), was also included. In addition, the perturbation differed between both experimental runs. In Experiment 1, a gradual temperature decrease was conducted by lowering the temperature from 37 to 17 °C at a rate of 2 °C per day and keeping the latter temperature constant during the last 35 operating days (around 2 hydraulic retention times). The temperature was modified by adjusting the temperature set point of the surrounding water bath. In Experiment 2, an abrupt perturbation (the temperature dropped from 37 to 17 °C (room temperature) in 24 h) was performed by turning off the water bath. After working at 17 °C for 10 days, the water bath was again connected at day 161 (the temperature of the reactor rose to 37 °C in one day) and maintained until the end of the Experiment 2. Moreover, the effect of using a different PM batch was also evaluated in Experiment 2, since typically the manure has different characteristics depending on the seasonal period.

2.3. Analytical methods

VFA (acetic, propionic, i-butyric, n-butiric, i-valeric and n-valeric) were analyzed by gas chromatography (HP, 5890A) equipped with a Flame Ionization Detector (HP, 7673A). COD, solids, TKN, ammonium, alkalinity and lipids were determined according to standard methods (APHA, 1998).

2.4. Molecular techniques

A selection of biomass samples were analyzed with DGGE plus sequencing, FISH and also 16s rRNA characterization with the Illumina MiSeq platform with the main focus of determining the microbial community dynamics during the perturbation and recovery periods. In this way, 21 samples from Experiment 1 (days 0, 15, 48, 66, 85, 95, 104, 124, 135, 145, 150, 152, 154, 157, 159, 161, 164, 171, 178, 185 and 194) and 14 samples from Experiment 2 (days 0, 49, 98, 150, 152, 155, 157, 161, 164, 168, 173, 181, 197 and 211) were selected.

2.4.1. Denaturing gradient gel electrophoresis and sequencing

DNA extraction, PCR, DGGE and sequencing were performed according to Regueiro et al. (2012), based on the primers U968-f and L1401-r for *Bacteria* and the primers A109 (T)-f and 515-r for *Archaea*. Cluster analysis was conducted using Bionumerics software v.6.1 (Applied Maths, Sint-Martens-Latem, Belgium).

2.4.2. Fluorescent in situ hybridization

Probe sequences and formamide concentrations were applied according to probeBase following the protocol explained by Regueiro et al. (2012). The probes used were: $Eub338_{mix}$ (*Bacteria*), CFX1223 (*Chloroflexi*), CFB562 (*Bacteroidetes*), LGC354 (*Firmicutes*), Arc915 (*Archaea*), Ms821 (*Methanosarcina*), Mx825 (*Methanosaeta)* and MB1174 (*Methanobacteriales*). The abundance of each population was qualitatively evaluated by DAIME program (Daims et al., 2006). At least six photos were taken per 20 μL of fixed sample.