Waste Management xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Changes in microbial community during hydrogen and methane production in two-stage thermophilic anaerobic co-digestion process from biowaste

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ARTICLE INFO

Article history:
Received 24 August 2015
Revised 11 January 2016
Accepted 14 January 2016
Available online xxxx

Keywords:
Biohydrogen
Thermophilic
Anaerobic co-digestion
Two phases
Biogas
Organic waste

ABSTRACT

In this paper, the microbial community in a two-phase thermophilic anaerobic co-digestion process was investigated for its role in hydrogen and methane production, treating waste activated sludge and treating the organic fraction of municipal solid waste. In the acidogenic phase, in which hydrogen is produced, *Clostridium* sp. clusters represented 76% of total *Firmicutes*. When feeding the acidogenic effluent into the methanogenic reactors, these acidic conditions negatively influenced methanogenic microorganisms: *Methanosaeta* sp., (*Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*) decreased by 75%, 50%, 38% and 52%, respectively. At the same time, methanogenic digestion lowered the numbers of *Clostridium* sp. clusters due to both pH increasing and substrate reduction, and an increase in both *Firmicutes* genera (non *Clostridium*) and methanogenic microorganisms, especially *Methanosaeta* sp. (208%). This was in accordance with the observed decrease in acetic (98%) and butyric (100%) acid contents. To ensure the activity of the acetate-utilizing methanogens (AUM) and the acetogens, high ratios of H_2-utilizing methanogens (HUM)/AUM (3.6) were required.

1. Introduction

In recent years, phase-separated anaerobic digestion (AD) process in a two-stage process has been widely used throughout Europe for the treatment of biowaste (Pavan et al., 2000; Schievano et al., 2012). This system is normally composed of an hydrolysis-acidogenesis step carried out in the first-phase (dark fermentation) and an acetogenesis–methanogenesis step carried out in the second phase (Zahedi et al., 2013a). The different growth rates and pH optima for hydrogen producing microorganisms (between 5.5 and 6.5) and methanogenic microorganisms (around pH 7), have led to the development of the two-stage AD process (De la Rubia et al., 2009). This approach has been used to the hydrogen production (HP) in the first phase reactor and methane production (MP) in the second phase reactor, with the final purpose of mixing the two gasses to achieve bio-Hythane (50–55% CH_4, 5–10% H_2 and 35–40% CO_2), a biogas that offers better combustion and reduced greenhouse gas emissions compared to fossil fuels (Cavinato et al., 2011; Liu et al., 2012).

Due to the advantages of the AD process, an ample research has been done on the optimization of AD for treating the organic fraction of municipal solid waste (OFMSW), including the interesting option of the co-digestion process. Benefits of co-digestion include: dilution of potential toxic compounds, improved balance of nutrients, synergistic effect of microorganisms, increased load of biodegradable organic matter and higher biogas yield (Callaghan et al., 1999). Hamzawi et al. (1998) and Sosnowski et al. (2003) found an average enhanced value of biogas production from the codigestion of wastewater treatment sludge and OFMSW.

Conventional bioconversion of waste activated sludge (WAS) and OFMSW in AD systems is usually characterized by hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first three steps are carried out by *Bacteria* domain, while the fourth step (methanogenesis) is undertaken by *Archaea* domain. *Bacteria* constitute a large domain of prokaryotic microorganisms. The majority of *Bacteria* identified in anaerobic digesters are covered by the following phyla: *Firmicutes* (Ariesyady et al., 2007), *Actinobacteria* (Ariesyady et al., 2007), *Spirochaetes* (Lee et al., 2013), *Bacteriodetes*

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http://dx.doi.org/10.1016/j.wasman.2016.01.016
0956-053X/

Nomenclature

AD	anaerobic digestion
COD	chemical oxygen demand
FISH	fluorescence in situ hybridization
GP	gas production
HP	hydrogen production
HRT	hydraulic retention time
MP	methane production
OFMSW	organic fraction of municipal solid waste
OLR	organic loading rate
rRNA	ribosomal RNA
SHP	specific hydrogen production
SMP	specific methane production
TKN	total Kjeldahl nitrogen
TP	total phosphorus
TS	total solids
VS	volatile solids
WAS	waste activated sludge
TVS	total volatile solid
VFA	volatile fatty acid
WAS	waste active sludge
WWTP	Waste Water Treatment Plant

and *Proteobacteria* (Chouari et al., 2005). *Clostridia* are a highly polyphyletic class of *Firmicutes*, including *Clostridium* and *Thermoanaerobacter* genera. They are obligate anaerobes capable of producing endospores. *Clostridium* sp. clusters are the predominant strains involved in the HP (M.J. Lee et al., 2009). On the other hand, *Archaea* constitute a domain of single-celled microorganisms. Methanogenic *Archaea* is a phylogenetically diverse group of strictly anaerobic *Euryarchaeota* with an energy metabolism that is restricted to the formation of CH_4 from CO_2 and H_2, formate, methanol, methylamines and/or acetate (Raskin et al., 1994). *Methanococcales*, *Methanobacteriales*, *Methanomicrobiales* and *Methanosaeta* sp. are considered to cover the majority of the methanogens encountered in anaerobic digesters (Yu et al., 2005; C. Lee et al., 2009). Only *Methanosaeta* sp. has the unique characteristic of relying on acetate as the sole energy source; the other three are H_2-utilizing methanogens (HUM).

Most researchers have focused on the optimization of gas production (GP), removal of organic matter and process optimization of two-stage AD process, but very few reports have discussed in detail the microbial population dynamics involved during the different stages of the thermophilic anaerobic co-digestion of WAS and OFMSW for hydrogen and methane production.

Fluorescence in situ hybridization (FISH) is a well established technique for the study of microbial populations. In FISH, fluorescently labelled oligonucleotide probes target and bind to ribosomal RNA (rRNA) providing an effective means of identification and qualitative and/or quantitative microbial population analysis in natural and engineered environments (Crocetti et al., 2006; Ariesyady et al., 2007; Montero et al., 2009; Zahedi et al., 2013a, 2013b, 2014a, 2014b).

In this paper the functional *Bacteria* and *Archaea* community structures in the substrates and in the acidogenic and methanogenic effluents of a two-stage thermophilic anaerobic co-digestion process treating WAS and OFMSW was investigated. The microbial community structures were quantitatively investigated using different specific probes employing FISH and consequently the results were related to process performance.

2. Materials and methods

2.1. Experimental equipment and operating conditions

Two laboratory-scale continuously stirred tank reactors were employed. The first reactor, dedicated to the HP (first phase, dark fermentation), had a 3.5 L working volume, while the second reactor (second phase) dedicated to the MP had a 18.5 L working volume. Both were heated by a hot water recirculation system and maintained in thermophilic conditions (55 °C). The system was fed semi-continuously, once per day, and the organic loading rates (OLR) in the first and second phase were 16 kg TVS/m^3 d and 3 kg TVS/m^3 d, respectively, while the corresponding hydraulic retention times (HRT) were 3 and 16 d for the first and second phase reactors, respectively. The whole experiment length was 70 d. After the start-up period (0–40 d), the stationary phase (41–70 d) was reached.

2.2. Substrate

The wastes used to feed the acidogenic reactor were collected from a wastewater treatment plant (WWTP) located in Treviso (northern Italy). The substrate employed in this study was a mix of OFMSW and WAS. The volume ratio OFMSW:WAS was 1:5, calculated in order to have an OLR of 16 kg TVS/m^3 d in the first phase reactor. The OFMSW and WAS were stored at −4 °C in order to avoid the degradation of the substrate by the microorganisms present in it. The OFMSW was reduced in size using a grinder and mixed with WAS. The feedstock was prepared daily and no pretreatment was implemented (i.e. chemical reagent or thermal treatment). The characteristics of the substrate in terms of total solids (TS), total volatile solids (TVS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) are shown in Table 1.

2.3. Analytical methods

The analytical determinations applied in this study can be grouped in two categories: physical–chemical analysis and microbiological analysis.

2.3.1. Physical–chemical analysis

The effluents of both the reactors were monitored 2–3 times per week in terms of TS, TVS, COD, TKN and TP. The process stability parameters, namely pH, volatile fatty acid (VFA) content and

Table 1
Substrate characterization.

Parameters	OFMSW		WAS	
	Avg.	Std. Dev.	Avg.	Std. Dev.
TS (g/kg)	283	29.3	27	1.1
TVS (g/kg)	234	21.9	20	1.0
COD (g/kg TS)	946	150	850	87
TKN (g/kg TS)	19.4	9.2	47.2	2.6
TP (g/kg TS)	7.4	3.0	17.4	1.6
pH	5.2	0.4	6.4	0.1
Alkalinity (pH = 6) (mg$CaCO_3$/L)	–	–	187.4	95.6
Alkalinity (pH = 4) (mg$CaCO_3$/L)	–	–	803.2	202.3
NH_4^+	–	–	133.3	45.9

Please cite this article in press as: Zahedi, S., et al. Changes in microbial community during hydrogen and methane production in two-stage thermophilic anaerobic co-digestion process from biowaste. Waste Management (2016), http://dx.doi.org/10.1016/j.wasman.2016.01.016