Bioresource Technology 190 (2015) 106–113

Contents lists available at ScienceDirect

Bioresource Technology

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

Biomass hydrolysis inhibition at high hydrogen partial pressure in solid-state anaerobic digestion

E.A. Cazier, E. Trably *, J.P. Steyer, R. Escudie

INRA, UR0050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, 11100 Narbonne, France

HIGHLIGHTS

- H_2 partial pressure inhibits specifically hydrolysis in mesophilic ss-AD.
- CO_2 is a limiting factor for CH_4 production in ss-AD through H_2 consumption.
- Microbial community is not impacted by H_2 inhibition.

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form 15 April 2015
Accepted 16 April 2015
Available online 22 April 2015

Keywords:
Dry anaerobic digestion
Gas transfer
Hydrogen
Hydrolysis
Lignocellulosic residues

ABSTRACT

In solid-state anaerobic digestion, so-called ss-AD, biogas production is inhibited at high total solids contents. Such inhibition is likely caused by a slow diffusion of dissolved reaction intermediates that locally accumulate. In this study, we investigated the effect of H_2 and CO_2 partial pressure on ss-AD. Partial pressure of H_2 and/or CO_2 was artificially fixed, from 0 to 1 557 mbars for H_2 and from 0 to 427 mbars for CO_2. High partial pressure of H_2 showed a significant effect on methanogenesis, while CO_2 had no impact. At high P_{H_2}, the overall substrate degradation decreased with no accumulation of metabolites from acidogenic bacteria, indicating that the hydrolytic activity was specifically impacted. Interestingly, such inhibition did not occur when CO_2 was added with H_2. This result suggests that CO_2 gas transfer is probably a key factor in ss-AD from biomass.

1. Introduction

Over the past decade, solid-state anaerobic digestion (ss-AD) also called dry anaerobic digestion or high-solids AD has gained a wide interest in Europe. Agricultural waste and organic fraction of municipal waste had been particularly used as substrates (Baere et al., 2010). In ss-AD, organic matter contained in the waste is biologically converted by anaerobic bacteria into a biogas composed of CH_4 (50–70%) and CO_2 (30–50%). Remaining organic matter called digestate can be further reused on land as fertilizer with several sanitary and environmental restrictions. The biogas produced by AD can be converted into electricity and heat by cogeneration or, after purification, can be used as biofuel (95% of CH_4) or directly injected in natural gas pipelines (Weiland, 2010).

Anaerobic digestion is composed of four microbial steps: first, organic matter (proteins, lipids and polysaccharides) is hydrolyzed into soluble molecules by extracellular enzymes excreted by hydrolytic microorganisms (Montero et al., 2008). Hydrolysis is mostly the limiting step of AD when solid organic matter is used as substrate (Pavlostathis and Giraldo-Gomez, 1991). The second step corresponds to acidogenesis: amino-acids, saccharides and fatty acids are transformed into volatile fatty acids (VFAs) such acetate, butyrate, propionate, or into others organics acids, such as lactate, or in alcohols, i.e. ethanol, butanol, in presence of fermentative microorganisms such as *Clostridium* sp. (Fritsch et al., 2008). The third step is called acetogenesis where all types of VFAs are transformed into acetate, CO_2 and H_2 by two types of microorganisms: (1) syntrophic acetogens, e.g. *Syntrophobacter wolinii* or *Syntrophomonas wolfei*, also called Obligate Hydrogen Producing Bacteria (OHPB), are converting VFAs, alcohols and fatty acids to H_2, CO_2 and acetate (Amani et al., 2010). These microorganisms are synthrophs of methanogens since all acetogenic reactions are thermodynamically not favorable and end-products accumulation can inhibit their own production, in particular hydrogen (2) non synthrophic homoacetogens, e.g. *Clostridium aceticum*, are using H_2 and CO_2 to produce acetate (Amani et al., 2010). In this case, the reaction is thermodynamically favorable and does not require the presence of methanogenic *Archaea*. Acidogenesis is also impacted by H_2 and CO_2 in wet AD and more specifically the production of propionate, butyrate and

* Corresponding author. Tel.: +33 (0) 468 425 172; fax: +33 (0) 468 425 160.
E-mail address: eric.trably@supagro.inra.fr (E. Trably).

http://dx.doi.org/10.1016/j.biortech.2015.04.055
0960-8524/

caproate (Arslan et al., 2012). Acetate and butyrate are concomitantly produced with H_2 and the consumption of these VFAs may be inhibited by high P_{H_2} (Ahring and Westermann, 1988; Ding et al., 2010; Hallenbeck, 2005). The last step corresponds to methanogenesis. Acetate, H_2 and CO_2 are transformed into CH_4 by two types of microorganisms: (1) acetotrophic methanogens using acetate as substrate and producing 70% of CH_4 in AD (Pavlostathis and Giraldo-Gomez, 1991) such as *Methanosaeta concilii* or *Methanosarcina acetivorans* (Amani et al., 2010), (2) hydrogenotrophic methanogens using CO_2 and H_2 as substrates, such as *Methanobacterium bryantii* or *Methanobrevibacter arboriphilus* (Amani et al., 2010).

Two main types of technologies have been developed to convert the solid organic matter in anaerobic digestion: wet AD and solid-state AD (ss-AD), with a Total Solids (TS) content below or higher than 15%, respectively (Baere et al., 2010). ss-AD presents several advantages such as reducing the water demand and lowering the costs related to water management, with a subsequent reduction of reactor size and less energy requirements for heating. These advantages contributed to a recent and large industrial development of ss-AD, with a rapid emergence of full-scale plants (Baere et al., 2010). Industrial digesters usually use agricultural, green or solid organic waste, under a TS content ranging from 15% to 30%. Since many industrial installations have been empirically developed, recent research has been devoted to optimize ss-AD processes through a better understanding of their limitations (Abbassi-Guendouz et al., 2012; Motte et al., 2013).

The key parameter driving the microbial processes in ss-AD is low water content since microbial end-products can locally accumulate and inhibit methanogenesis (Abbassi-Guendouz et al., 2012). In such systems, water availability presents two distinct forms: (1) "free water" that can act as a solvent for salts and soluble compounds (Pommier and Chenu, 2007), (2) "bound water" that has more structural bonding (chemical and physical interactions) than liquid or free water and thus, is unable to act as a solvent. The form of water depends mainly on the structure and composition of the organic matter structure. Moreover, when the TS content increases, the quantity of free water decreases (García-Bernet et al., 2011) and the transport of soluble content within the substrate can become a limiting factor (Bollon et al., 2013). Microbial activity can be impacted by water availability and methane yields varies significantly between wet and ss-AD, according to the nature of the substrate. Brown et al. (2012) reported that CH_4 yields of paper decreased from 19.2 L/kg TS to 8.7 L/kg TS in wet and ss-AD, respectively. In contrast, no CH_4 yields variation was observed with wheat straw, i.e. 11.6 L/kg TS (wet AD) and 12 L/kg TS (ss-AD). Overall, by increasing the TS content, substrate degradation and consequently the biogas production are reduced (Abbassi-Guendouz et al., 2012; García-Bernet et al., 2011; Motte et al., 2013). As an illustration, a decrease of organic waste degradation by 17% was observed when changing the TS content from 20% to 30% (Fernández et al., 2008).

When the water content decreases, the rheological behavior of the substrate is also modified (García-Bernet et al., 2011). Therefore, the diffusion of metabolic compounds within the substrate at a macroscopic level is lowered (Bollon et al., 2013). The limitation of ss-AD might be explained by the slow diffusion of dissolved inhibitory products inside the organic matrix generating local accumulation at microbial scale (Martin, 2001; Staley et al., 2011). In a recent study, Abbassi-Guendouz et al. (2012) suggested that methanogenesis inhibition at high solids content was mainly caused by gas transfer limitation and more particularly by a local accumulation of gases (H_2 and CO_2) leading to VFAs accumulation. H_2 has a well-described inhibitory effect on wet AD but the exact effect of H_2 on ss-AD remains unknown.

The objective of this study was to investigate the effect of dissolved gases on ss-AD. The accumulation of H_2 and CO_2, as by-products of acidogenesis, was investigated in particular since local accumulation of these gases may impact the overall ss-AD process. Experiments were designed and carried out to evaluate the effect of the partial pressure of H_2 alone or mixed with CO_2 on the overall reaction of ss-AD.

2. Methods

2.1. Substrate preparation

Wheat straw (*Triticum aestivum*) was used as model substrate representing lignocellulosic agricultural waste for its well-known composition in hemicelluloses, cellulose and lignin (Vassilev et al., 2012). Particles were fractionated in a cutting miller through a 1 mm grid, and further sieved between grids of 1 mm and 400 μm. Wheat straw particles had a TS content of 95%.

2.2. Operating conditions of the batch tests

Granular sludge originated from an industrial UASB reactor treating sugar factory effluents was used to inoculate the batch reactors. The sludge was mixed during 24 h at 35 °C to break the granules, and then centrifuged at 7841*g* (20 min, 4 °C) to obtain a microbial anaerobic inoculum with a TS content ranging between 10% to 13%. Final pH of the inoculum was 7.8.

A substrate/inoculum biomass (S/X) ratio of 3 (in volatile solids basis) was used as suggested elsewhere (Liew et al., 2012). The initial TS content of the flask was fixed at 25% where no inhibition of AD was reported (Abbassi-Guendouz et al., 2012; Motte et al., 2013). Wheat straw and inoculum were added in a S/X ratio of 3 and TS content was adjusted with the buffer solution after addition of trace elements. At this TS content, A solution of trace elements ($FeCl_2$ 2 g/L, $CoCl_2$ 0.5 g/L, $MnCl_2$ 0.1 g/L, $NiCl_2$ 0.1 g/L, $ZnCl_2$ 0.05 g/L, H_3BO_3 0.05 g/L, Na_2SeO_3 0.05 g/L, $CuCl_2$ 0.04 g/L, Na_2MoO_4 0.01 g/L) was added with a volume of 0.2 mL by flask. A buffer solution of sodium bicarbonate (0.0026 g $NaHCO_3$/g substrate) was added at the beginning to keep the pH around 7–8.

First, the medium (wheat straw, trace elements, buffer and inoculum) was introduced into a 3 L reactor operated during 10 days at 35 °C. This reactor was flushed with N_2 to avoid the presence of O_2. This pre-culture was performed to reach an active phase of methanogenesis and homogenize the substrate. Then, 20 g of the pre-culture was distributed in a thin layer (<1 cm) at the bottom of a 600 mL flask. Such thin layer was used to minimize the influence of the gas diffusion within the medium. The flasks were flushed with N_2 before the addition of other gases (H_2 alone or mixed with CO_2). Whatever the gas added, the initial pressure was around 1.5 bars. The flasks were then incubated during 13 days at 35 °C to evaluate the effect of the partial pressure of H_2 and/or CO_2 on the initial degradation of wheat straw in ss-AD.

Experiments were conducted in five separate runs, with three to four replicates for each condition. An experimental design plan was performed in order to investigate the partial pressure of H_2 (P_{H_2})

Table 1
Gas partial pressure investigated in different runs.

Runs	Partial pressure of H_2 (mbars)	Partial pressure of CO_2 (mbars)
1	0, 55, 105, 485 and 895	0
2	0, 650, 750, 932 and 1 020	0
3	0, 778 and 1 418	0
4	0, 828 and 1 545	0
5	0, 780 and 1 060	0, 327 and 427