Bioresource Technology 171 (2014) 350–359

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial community structure and dynamics during co-digestion of whey permeate and cow manure in continuous stirred tank reactor systems

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HIGHLIGHTS

- Community of *Bacteroidetes*, *Firmicutes*, *Methanobacteriales* and *Methanomicrobiales*.
- High feeding of whey led to bioreactors operating at the edge of stability.
- Parallel biogas reactors differed in performance and microbial communities.

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 21 August 2014
Accepted 22 August 2014
Available online 30 August 2014

Keywords:
Anaerobic digestion
Methane
Microbial community
High throughput sequencing
Co-digestion

ABSTRACT

Microbial community profiles in two parallel CSTR biogas reactors fed with whey permeate and cow manure were investigated. The operating conditions for these two reactors were identical, yet only one of them (R1) showed stable performance, whereas the other (R2) showed a decrease in methane production accompanied by accumulation of propionic acid and, later, acetic acid. This gave a unique opportunity to study the dynamics of the microbial communities in two biogas reactors apparently operating close to the edge of stability. The microbial community was dominated by *Bacteroidetes* and *Firmicutes*, and the methanogens *Methanobacteriales* and *Methanomicrobiales* in both reactors, but with larger fluctuations in R2. Correlation analyses showed that the depletion of propionic acid in R1 and the late increase of acetic acid in R2 was related to several bacterial groups. The biogas production in R1 shows that stable co-digestion of manure and whey can be achieved with reasonable yields.

1. Introduction

In the face of global challenges such as fossil fuel depletion, increasing greenhouse gas emissions and climate change, anaerobic digestion of organic material to biogas has become an attractive strategy for renewable energy production and sustainable waste disposal. Agricultural residues, municipal solid waste and wastewater have traditionally been the main substrates for biogas production. In addition, various residuals from the food processing industry have been proposed as possible substrates for biogas generation, including by-products from the dairy industry (Luo and Angelidaki, 2013).

Cheese whey is a by-product from cheese production. Between 115 and 160 million tons of whey are generated globally every year, half of which is transformed into food products or utilized for ethanol fermentation, while the rest is disposed (Guimarães et al., 2010). Due to its world-wide availability and high carbohydrate content, whey is considered a suitable substrate to produce biogas via anaerobic degradation. Whey proteins have a relatively high value and are typically removed from whey by ultrafiltration. Thus, it is mainly the whey permeate, i.e., a solution primarily composed of water, lactose and salts, that is available for anaerobic digestion. Co-digestion of whey permeate with cow manure or poultry waste has caught some interest because the latter feedstocks provide buffer capacity, nitrogen, and nutrients (Gelegenis et al., 2007). Furthermore, to prevent rapid growth of acid forming bacteria, the easily degradable lactose in whey permeate needs to be balanced by more recalcitrant substrates, such as lignocellulosic material in cow manure.

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http://dx.doi.org/10.1016/j.biortech.2014.08.095
0960-8524/

Anaerobic degradation of organic compounds to biogas is carried out by a relatively undefined microbial culture which varies according to its origin, substrate composition, operational conditions and environmental parameters. Generally, anaerobic degradation proceeds via four main steps: hydrolysis, fermentation, anaerobic oxidation and methanogenesis. The three first steps are carried out by a large consortium of bacteria, while specialized groups of methane producing archaea (methanogens) are responsible for the final step (Gujer and Zehnder, 1983). Efficient and successful conversion of organic matter to biogas relies on a close and balanced cooperation between the different groups of microorganisms.

The bacteria degrade organic substrates to acetate and longer-chain volatile fatty acids (VFAs), mainly propionate and butyrate, in addition to lactate and alcohols (hydrolysis & fermentation). Acetate can be directly utilized in the aceticlastic methanogenesis or in a two-step pathway comprised of acetate oxidation to hydrogen and carbon dioxide by syntrophic acetate oxidizing bacteria (SAOB) and a subsequent conversion of these products to methane by hydrogenotrophic methanogens (Schnürer et al., 1999). The other reduced intermediate compounds, including long-chain VFAs, must be oxidized to acetate, formate or hydrogen prior to methanogenesis. This anaerobic oxidation process, also called acetogenesis, is carried out by acetogens. Degradation of VFAs is generally thermodynamically unfavorable ($\Delta G^{\circ\prime} < 0$), and the conversion is only possible if the hydrogen partial pressure is kept low, e.g., by hydrogen consuming methanogens. Interspecies hydrogen/formate transfer between sulfate reducing bacteria and methanogens is also widespread in nature, and may play an important role in degradation of long chain fatty acids (Schink, 1997). Thus, a syntrophic cooperation between bacteria and methanogens is critical for the process. The low growth rate and sensitivity to toxic compounds of methanogenic archaea compared to that of bacteria makes the complex microbial community in a biogas digester vulnerable to changes in operational parameters. For example, an increase in the organic loading rate of the system will speed up the hydrolysis-acidification process, whereas hydrogenotrophic methanogens may fail to consume all the hydrogen produced, thus leading to accumulation of reduced metabolites, such as VFAs.

The start-up phase of biogas reactors is considered as the most critical period, and comparisons of the microbial diversity in anaerobe reactors during start-up and steady-state conditions have revealed considerable shifts in community composition (Cardinali-Rezende et al., 2012). Generally, studies of biogas microbial communities have focused on stably performing reactors (e.g., Pope et al., 2013) and only a few recently published studies have explored the dynamic changes in microbial communities in response to instability and changes during the digestion period (Westerholm et al., 2012; Ziganshin et al., 2013). Here, the community dynamics in two parallel continuously stirred tank reactors (CSTR) co-digesting cheese whey permeate and cow manure was investigated. 16S rRNA gene sequencing was used to characterize the dynamics of the microbial community over a period of three months. Both reactors were continuously analyzed with regard to biogas production and accumulation of volatile fatty acids. Although the two reactors were technical parallels and ran under identical conditions, they differed in performance over time. This gave a unique opportunity to study the dynamics of the microbial communities in two biogas reactors apparently operating close to the edge of stability.

2. Methods

2.1. Substrate and inoculum

Whey permeate obtained by ultrafiltration of whey was supplied by TINE SA, Norway. The dry matter content (DM) was 16.2% of which 90.8% were volatile solids (VS). Manure (11.3% DM and 85.9% VS) was supplied by the Department of Animal Sciences, Norwegian University of Life Sciences, Norway. These feedstocks were stored at 4 °C until required for the digestion experiments. The microbial inoculum for this study was obtained from a local biogas plant (Tomb Biogass, Norway), that runs large scale continuous anaerobic digestion of cow manure and food waste at mesophilic temperature (37 °C; pH 7.6). The DM content of the inoculum was 5.2% and VS was 68.5%. Prior to the experiments, the inoculum was incubated anaerobically (37 °C, 10 days) to reduce endogenous biogas production in the subsequent experiments. Chemical composition data for the whey, manure and inoculum is given in Table 1.

2.2. CSTR experiments and biogas production

Anaerobic digestion was carried out in laboratory-scale (10 L) continuously stirred tank reactors (CSTR, Dolly, Belach Bioteknik, Stockholm, Sweden) with a working volume of 6 L. Two parallel reactors (R1 and R2) for were filled with inoculum (6 L). Initially each reactor was fed with 0.5 g VS/L/day of a mixture of whey and manure (48.0% VS from manure). It was then gradually increased day by day to reach the final organic loading rate of 2.7 g VS/L/day 10 days after the first feeding, and the loading rate was then kept constant. Because accumulation of VFAs was observed, the whey/manure ratio in the feed was reduced after 44 days of operation. For R1 the feed was changed to 2.9 g VS/L/day (64.2% VS from manure) and kept constant for the rest of the period. R2, which showed a more severe accumulation of VFAs, was run in the following way: day 44–57, only manure, 1.9 VS/L/day; day 58–79, whey and manure, 2.9 g VS/L/day (64.2% VS from manure); day 80 to the end of the experiment, only manure, 1.9 VS/L/day. The operational conditions of the reactors were: 37 °C, initial pH 7.5, 180 rpm, and feeding 6 days a week with a hydraulic retention time (HRT) of 25 days. The HRT was kept constant by adding water to the substrate mixture. See Fig. S1 for a schematic diagram of experimental setup.

Continuous real-time monitoring of pH, stirrer speed, temperature, gas flow and gas volume produced was managed using the BIOPHANTOM© software (Belach Bioteknik, Stockholm, Sweden). Produced biogas was measured monitoring volume displacement in dedicated glass columns. Based on methane concentration and biogas volume the ideal gas law was used to calculate methane production.

2.3. VFA analysis

Samples for Volatile fatty acids (VFAs) determination were collected once a week, and stored at −20 °C before analysis. VFAs (e.g.,

Table 1
Chemical characteristics of the materials. Volatile Solids (VS) and elements are expressed as percentage of Dry Matter (DM). Oxygen content was calculated by subtracting C, N and H values from VS content.

Substrate	Total C (%)	Total H (%)	Total N (%)	Total O (%)	Dry matter (%)	Volatile solid (%)	pH
Whey	41.1	5.3	0.4	44.0	16.2	90.8	7.2
Manure	45.2	5.6	1.1	34.0	11.3	85.9	7.3
Inoculum	32.7	4.0	2.8	29.0	5.2	68.5	7.6