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Biogas upgrading by injection of hydrogen in a two-stage Continuous Stirred-Tank Reactor system

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Abstract
An innovative method for biogas upgrading (i.e. CH4 content more than 90%) combines the coupling of H2, which could be produced by water electrolysis using surplus renewable electricity produced from wind mills, with the CO2 of the biogas. CO2 is biologically converted to CH4 by hydrogenotrophic methanogens. In this study, a novel serial biogas reactor system is presented, in which the produced biogas from the first stage reactor was introduced in the second stage, where also H2 was injected. The effects of the H2 addition on the process performance and on the microbial community were investigated. It was shown that after the H2 addition, the CH4 rate increased by 45%, resulting in an average CH4 content of approximately 85%, with a maximum of 93.9%. The increase of the pH to 8.5, due to the CO2 conversion, was not an inhibitory factor, demonstrating the adaptation of microorganisms to these pH levels. The profiles of the microbial communities prior and after the H2 addition showed distinct differences. Changes in the archaeal community and more specifically increase in the relative abundance of Methanobrevibacter sp. and Methanoculleus sp. indicated that the methanogenic pathway was clearly shifted from aceticlastic to hydrogenotrophic.

Keywords
Biogas upgrading; hydrogen; anaerobic digestion; hydrogenotrophic methanogens

INTRODUCTION
The exploitation of biomass and wind, as renewable energy sources, is of a great importance for the Danish Energy policy. Biogas produced by anaerobic digestion (AD) of biomass contains approximately 60% CH4 and 40% CO2. Upgrading of biogas to a CH4 content higher than 90% increases its heating value and makes it usable as an alternative to natural gas (Ryckebosch et al. 2011). Additionally, up to 40% of the electricity from wind is a temporary surplus and although some of it is exported to neighboring countries (Sharman 2005), the potential of wind mills is not fully utilized. An attractive way to exploit this surplus is to electrolyze water to produce H2, and then to combine this H2 with the CO2 of the biogas for biogas upgrading. Previous studies have shown that this biological conversion is feasible (Luo et al. 2013a). Hydrogen-mediated CO2 conversion to CH4 may, thus, be an attractive alternative that could be implemented using the existing energy infrastructures for the biogas plants. Moreover, this concept could reduce upgrading costs and offer the possibility of electricity production according to the energy demand variation. AD is a complex process carried out by several microbial groups. In particular, in H2 mediated upgraded reactors, CO2 is converted to CH4 by hydrogenotrophic methanogens (Luo et al. 2013a). Our hypothesis was that the H2 addition could markedly modify the microbial community, causing an imbalance in the methanogenic pathway and increasing the CO2 consumption. Nevertheless, during this process, the pH of the system could significantly increase because of the CO2 removal, affecting negatively the AD process. Moreover, gas-liquid mass transfer represents a limitation of the process (Guiot et al. 2011) because the H2 must be dissolved in the liquid phase of the reactor to be utilized by microorganisms. The aim of the present study was to investigate the effect of the H2 addition in a continuous-flow stirred-tank reactor (CSTR) system, composed by two connected CSTR in series. The produced biogas from the first stage reactor was introduced along with H2 to the secondary reactor. To determine microbial relative abundance changes upon the hydrogen addition...
addition, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR–DGGE) and 16S rDNA gene next generation sequencing (NGS) were performed on the microbial community at steady states of the reactor before and after the H2 addition.

MATERIALS AND METHODS

Substrate characteristics and preparation of the feedstock

The raw cattle manure used in the experiment was obtained from Hashøj biogas plant, Denmark. After arrival, the manure was sieved to remove large particles and barley and stored at -20°C. The frozen manure was kept at 4°C for 2–3 days before use. The manure had a pH of 7.44, total solids (TS) and volatile solids (VS) content of 47.40 and 34.56 g/L, respectively. The total Kjeldahl Nitrogen (TKN) and ammonium nitrogen were 3.03 ± 0.10 and 2.07 ± 0.01 g-N/L, respectively. The concentration of total volatile fatty acids (VFA) was 6.83 ± 0.48 g/L.

Continuously fed reactor setup, operation and analytical methods

The experiment was conducted in a two-stage CSTR system, with a total working volume of 3.5 L. The working volume of the primary reactor and secondary reactor were 1.5 L and 2 L, respectively. The reactors were mixed by magnetic stirrers and the temperature was maintained steady at 55°C using thermal jackets. The hydraulic retention time (HRT) for the primary and the secondary reactor were 15 days and 20 days, respectively. The primary reactor was fed twice per day with cattle manure, while the secondary was fed with the effluent from the primary. Once steady state conditions were recorded, H2 was continuously injected to the secondary reactor, through a diffuser placed at the bottom of the reactor. The H2 flow rate was set to be 4 times the CO2 production before the H2 addition. TS, VS, NH3–N and TKN, were analyzed according to the Standard Methods for Examination of Water and Wastewater (APHA 2005). Samples from both reactors were taken for pH and VFA measurements twice per week. VFA concentration and biogas composition was determined by gas chromatography, as described by Kougias et al., (2013). The biogas production was recorded in daily basis.

Microbial Community Composition

DNA was extracted from the secondary reactor using the RNA PowerSoil® DNA Elution Accessory Kit, according to the manufacturer’s instructions. Changes in microbial abundance were determined by PCR-DGGE analysis on 16S rDNA gene V4 hypervariable region with universal primers (Caporaso et al. 2012) and on V2-V3 regions specifically for the archaeal community. PCR-DGGE and sequence analysis were done according to Luo & Angelidaki 2013a. The 16S rDNA gene NGS was done using Illumina 2000 sequencing technology.

RESULTS AND DISCUSSION

Process monitoring and biogas upgrade

Before the H2 addition, the produced biogas from the CSTR system contained approximately 67% CH4 and 33% CO2. After the H2 addition, the CH4 rate increased by 45% (i.e. from 247 mL/day to 359 mL/day) and the CO2 rate decreased by 77% (i.e. from 121 mL/day to 28 mL/day), due to the conversion of CO2 to CH4. Consequently, the average CH4 content increased to 85.1%, with a maximum of 93.9%, and the average CO2 content decreased to 6.6%, with a minimum of 5.6%. Unfortunately, not all the injected H2 reacted with the CO2 during most of the experimental period, as an average H2 content of 8.3% was still present in the biogas. The incomplete utilization of H2 could be due to the low hydrogen gas-liquid mass transfer rate (Guiot, Cimpoia, and Carayon 2011). This implies that the distribution of the H2, through the diffuser’s pores, was not sufficient enough to fully solubilize the injected H2. Problems with insufficient distribution of the H2 through a
diffuser were also previously identified by Luo et al. (2013b). Although, in correspondence to the maximum CH4 content, no H2 was detected, unconverted CO2 was still present in the biogas. The incomplete conversion of the CO2, even with a complete utilization of the H2 may be due to the production of additional biogas by residual organic material or to a surplus contribution of CO2 by the bicarbonate contained in the inoculum. The pH and VFA levels of the primary reactor remained stable for the whole period. In the secondary reactor, VFA levels were significantly low (i.e. less than 0.6 g/L) during the whole experiment. Conversely, upon the H2 addition, the pH of the secondary reactor increased from 7.89 to 8.49. It is known that methanogenesis occurs in a pH range from approximately 5.5 to 8.5 and the process can be severely inhibited if the pH is below 6 or above 8.3 (Angelidaki et al. 2011). Despite the buffer capacity of the cattle manure, the addition of H2 to the reactor could increase the pH, inhibiting the microorganisms’ growth and activity (Mu et al. 2006). Nevertheless, in this experiment no inhibition of the process and decrease in the conversion of CO2 to CH4 occurred. To support our findings, we performed a batch assay experiment with inoculum coming from the secondary reactor, at different pH values. The results from the batch experiment showed that CH4 production was still detected at pH 8.5, which could have been possible due to the adaptation of microorganisms to the increased pH conditions.

**Microbial Community Composition**

The microbial relative abundance before and after the H2 addition was determined using PCR-DGGE analysis. The profiles of the microbial communities showed several distinct bands representing the dominant microbial phylogenetic groups (Figure 1A) and the dominant archaea (Figure 1B) populating the reactor at steady state conditions. In accordance with De Francisci et al. (2015), BLAST results indicated that dominant phyla had high similarity (>89%) to uncultured Bacteroidetes (EU639239) and Clostridia (Firmicutes phylum; JQ897459; KJ650763). More specifically, the microorganisms belonging to the bands A and C were identified as member of the class *Flavobacteria*, with 78% of similarity to *Blattabacterium* sp. (NR102962). Bands D and E were, instead, identified at family level, with respectively 89% and 88% of similarity to *C. clariflavum* (NR102987) and *C. cellobioparum* (NR113360). Band B was identified at phylum level as uncultured *Actinobacterium* (77% of identity). *Bacteroidetes* decreased in the samples taken after the H2 addition, conversely *Clostridium* and *Actinobacterium* increased after the H2 addition. The dominance of these phyla could be explained due to their important role in the hydrolysis of polysaccharides present in the biomass (Stolze et al. 2015). It should be noted that the limitation in the taxonomic assignment in biogas reactors was also previously reported and is due to the complexity of the microbial community (De Francisci et al. 2015). Moreover, it was found that the archaeal community both prior and after H2 addition represented just a minority of the reactor population. The bands A, D and E were identified at genus level, with similarity higher than 96% to *M. smithii* (NR_074235.1), *M. thermophilus* (NR_028156.1) and *M. bourgens* (NR_042786.1), respectively. Band B was identified as member of the Euryarchaeota, with 75% of similarity to *M. formicicum* (NR_115168.1). Finally, band C was identified to *Methanosarcinaceae*, with an identity of 92%. The results from the DGGE analysis for the archaeal community were consistent with the reactor operation and validated our initial hypothesis that the methanogenic pathway would shift from acetoclastic to hydrogenotrophic after the H2 addition. More specifically, the abundance of the hydrogenotrophic methanogens (i.e. *Methanobrevibacter* sp., *Methanoculleus* sp. and the archaea with low similarity to *Methanobacterium*) increased after the H2 addition, with a concomitant decrease in the abundance of the acetoclastic *Methanosarcinaceae* sp. Finally, the high relative abundance of the *Methanoculleus* sp. similar to *M. thermophilus*, prior to the H2 addition was in accordance with previous studies. Chachkhiani et al. (2004) reported that the dominant archaeal species taking part in the thermophilic anaerobic bioconversion of cattle manure to CH4 and CO2 were *M. thermophilus* and *M. thermophila*. Finally, it should be noted that the results from 16S
rDNA gene NGS, which will be available during the conference, will provide a more detailed overview of the microbial communities populating the CSTR system prior and after the H2 addition.

![Figure 1](image1.png)

**Figure 1.** Technical replicates of DGGE profiles; a) bands corresponding to the most abundant microorganisms of the community and b) bands corresponding to the archaeal community.

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