Science of the Total Environment 595 (2017) 912–919

Contents lists available at ScienceDirect

Science of the Total Environment

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

Characteristics of adapted hydrogenotrophic community during biomethanation

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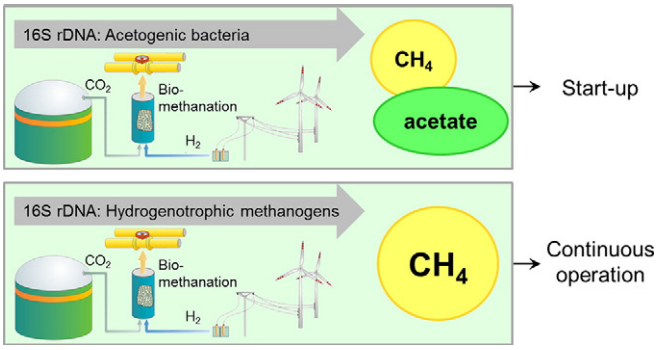
HIGHLIGHTS

- Acetate accumulation during biomethanation start-up results in reduced CO_2 conversion.
- Acid tolerance and product formation evaluated for different microbial consortia.
- 16S rRNA gene sequencing proved altered composition after hydrogenotrophic enrichment.
- Acetate accumulation linked to presence of ~50% acetogenic bacteria during start-up.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form 7 March 2017
Accepted 8 March 2017

Keywords:
Acetic acid
Anaerobic digestion
Methanongenic Archaea
Biogas
16S rRNA gene sequencing

ABSTRACT

The results presented in this study were carried out as concomitant experiments during the start-up and operation of a biomethanation unit to evaluate the effect of process parameters on carbon conversion, product formation (methane and acetate) and community composition. For that, two different samples were withdrawn from a trickle-bed reactor with immobilized enrichment culture of hydrogenotrophic methanogens adapted from sewage sludge. One sample was taken from the recirculation liquid during start-up phase while the other was withdrawn directly from the carrier material in the reactor. Elevated acid levels especially during start-up were shown to affect the overall carbon conversion. This effect was also seen during the acid tolerance testing reported here. Final acid concentrations of 1.6 ± 0.3 g/L resulted in a reduced conversion ratio of only 46%. Without acid addition complete conversion of CO_2 in the headspace was achieved. However, maximum methane production of 0.55 ± 0.02 mmol after 4 days of incubation was monitored at moderate initial acetate concentration of 0.4 g/L. In both analyzed inoculation materials *Methanobacterium* species were by far the most dominant Archaea with 21.8% in the recirculation liquid during start-up and 84.8% in the enrichment culture immobilized on the carrier material. The microbial composition of the two analyzed samples is in accordance with the results obtained for the carbon conversion and product formation. With approximately 50% of *Bacteroidetes* and *Firmicutes* present during reactor start-up the acetic acid production significantly contributed to the overall carbon conversion. In contrast, methane was produced almost exclusively in trials representing continuous operation where acetogenic bacteria accounted only up to 17.5%. In summary, the acid accumulation monitored during reactor start-up of a

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http://dx.doi.org/10.1016/j.scitotenv.2017.03.074

biomethanation unit is most likely to result from the microbial composition present. Nevertheless, complete adaptation to hydrogenotrophic conditions was proven to alter the consortium and yield methane as main product alongside high carbon conversion of up to 70.5 ± 1.8%.

1. Introduction

Biogas from anaerobic digestion of various organic sources has a relevant role in the renewable energy mix. Within the Power-to-Gas concept biomethane can act as intermediate storage for surplus electricity from wind- and solar power. Biomethanation of CO_2 and H_2 is of increasing interest (Rittmann, 2015) as the thermo-chemical alternative, the Sabatier process, has several limitations. Compared to thermochemical conversion the biological process consumes significantly less energy due to the mild conditions (ambient pressure, moderate temperatures) applied (Song et al., 2004). Moreover, biological methanation shows higher tolerance against gas impurities which are typically found in the feed gases as no sensitive Ni-/Ru-catalysts are required (Götz et al., 2014). The presence of certain chemical species (specifically sulfur) in the feed gas might interfere or even deactivate the catalyst material used in the Sabatier process. In contrast, biological methanation provides stable carbon conversion alongside a robust process showing high resistance to trace components in the gas stream without additional cleaning steps required. Against the background of a CO_2 emission-driven climate change industrial off-gases containing significant amounts of CO_2 become increasingly interesting as alternative carbon source.

Biomethanation may occur via two pathways:

a) direct conversion of H_2 and CO_2 by hydrogenotrophic Archaea

b) indirect conversion involving two different microbial groups, homoacetogenic Clostridia producing acetate via the Wood-Ljungdahl pathway (syntrophic acetate oxidation) and acetoclastic Archaea which further convert this acetate into methane.

Biological biogas upgrading has already been proven to obtain methane concentrations suitable for grid injection. Still, biogas upgrading capacity needs still to be improved. Many investigations involve pure cultures where the pathway is implicitly known (De Vrieze et al., 2012; Martin et al., 2013; Rittmann et al., 2015). However, for a practical application unsterile operation with a self-establishing microbial community is a prerequisite. Mixed adapted cultures are more robust and do not require sterile conditions.

To better understand microbial community behavior the distribution of the different species is of interest. Possibly occurring side reactions in the upgrading system might be explained as the established culture originates from a diverse mixture of various organisms including Clostridia species and other bacterial strains involved in the metabolism of sulfur and nitrogen compounds etc. present in biogas sludge (Westerholm et al., 2016). During a change in conditions (substrate feeding, temperature or pH) the best adapted organisms have a clear advantage. If an organism is fast in utilizing the provided substrate it will overgrow the residual consortium and predominantly occur. However, other species than the dominantly occurring can still survive either due to spore formation or utilizing organic matter resulting from residual biomass. Hence, even heterotrophic strains can still be present under hydrogenotrophic conditions without any additional C-source besides CO_2. Depending on the conditions a wide variety of Bacteria and Archaea species might still be present in an adapted consortium originating from biogas sludge (Liu et al., 2009).

Since species representing both CO_2 utilizing pathways, homoacetogenic bacteria and hydrogenotrophic archaea, might still be present it is difficult to exactly yield the targeted product, in this case methane. The influence of various operational parameters on the conversion of H_2 and CO_2 by a mixed consortium can be significant. A fine adjustment of operation conditions is therefore crucial to adjust the consortium composition and hence, maximize the methane yield.

As microorganism strains derived from diverse environmental samples as present in sewage sludge are often difficult to cultivate, molecular approaches like the analysis of the 16S rRNA gene sequence crucially improved the characterization of such communities (Hofman-Bang et al., 2003). The chosen metagenomic approach allows evaluating the genetic potential of these microbial communities (Heyer et al., 2015; Nettmann et al., 2008).

The results presented in this study were carried out as concomitant experiments during the start-up and operation of a trickle-bed reactor for biomethanation by an adapted hydrogenotrophic consortium reported elsewhere (Rachbauer et al., 2016). Especially during the start-up phase of this trickle-bed reactor volatile fatty acids accumulation occurred, either as a result of insufficient microbial adaptation or due to the influence of operational conditions.

Negative influence of acid addition to moderately acid-tolerant methanogens on CH_4 formation was already proven for formate, acetate, propionate and butyrate (Horn et al., 2003). For stable biomethanation resulting in constant high gas quality it is important to establish a robust microbial culture. However, it was never evaluated how increased amounts of acetate affect the metabolism of an adapted hydrogenotrophic consortium with regard to product formation and carbon conversion.

Hence, this study evaluated the effect of H_2 partial pressure and acetic acid concentration on the carbon conversion of CO_2 to methane and acetate for two different samples. Microbial consortia represent the dominating consortium either during start-up phase or stable operation conditions of a trickle-bed reactor for biological methanation of biogas.

Furthermore, the microbial adaptation itself was studied. For that the microbial diversity of both hydrogenotrophic enrichment cultures was analyzed by 16S rDNA Illumina sequencing.

2. Material and methods

2.1. Inoculation material

For trials reported here a hydrogenotrophic enrichment culture was withdrawn from a continuously operated trickle-bed reactor fed with a gas mixture of pure biogas and bottled H_2. Gas was supplied from the bottom and nutrient solution was recirculated via a peristaltic pump via a drip funnel at a rate of 250 mL/min out of a 2.0 L reservoir placed in a water bath. The trickle-bed reactor consisted of two concentric glass columns with an inner diameter of 0.08 m and had a packed volume of 0.00578 m^3. Polypropylene packing rings (Hiflow rings type 15–7, RVT Process Equipment, Germany) served as the carrier material for the biofilm, offering a high specific surface of 313 m^2/m^3 at a void fraction of 91%.

The exact reactor set-up and operational conditions are reported elsewhere (Rachbauer et al., 2016). On the one hand inoculation material was withdrawn from the recirculation liquid (R) right after inoculation with sludge from an anaerobic digester and on the other hand biomass was also washed directly from the carrier material immobilized inside the reactor (C) representing the start-up phase and continuous operation, respectively. As it was not possible to open up the reactor during the experimental period, the consortium representing an established biofilm during stable operation (sample C) had to be flushed