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Biotic and abiotic dynamics of a high solid-state anaerobic digestion box-type container system

Andreas Walter [a,b,*], Maraike Probst [a], Stephan Hinterberger [c], Horst Müller [c], Heribert Insam [a]

[a] Institut für Mikrobiologie, Universität Innsbruck, Technikerstraße 25d, 6020 Innsbruck, Austria
[b] Hochschule Hamm-Lippstadt, Biotechnologie, Marker Allee 76-78, 59063 Hamm, Germany
[c] Müller Abfallprojekte GmbH, Hauptstraße 34, 4675 Weibern, Austria

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ABSTRACT

A solid-state anaerobic digestion box-type container system for biomethane production was observed in 12 three-week batch fermentations. Reactor performance was monitored using physico-chemical analysis and the methanogenic community was identified using ANAEROCHIP-microarrays and quantitative PCR. A resilient community was found in all batches, despite variations in inoculum to substrate ratio, feedstock quality, and fluctuating reactor conditions. The consortia were dominated by mixotrophic *Methanosarcina* that were accompanied by hydrogenotrophic *Methanobacterium*, *Methanoculleus*, and *Methanocorpusculum*. The relationship between biotic and abiotic variables was investigated using bivariate correlation analysis and univariate analysis of variance. High amounts of biogas were produced in batches with high copy numbers of *Methanosarcina*. High copy numbers of *Methanocorpusculum* and extensive percolation, however, were found to negatively correlate with biogas production. Supporting these findings, a negative correlation was detected between *Methanocorpusculum* and *Methanosarcina*. Based on these results, this study suggests *Methanosarcina* as an indicator for well-functioning reactor performance.

1. Introduction

The increasing production of waste is a natural consequence of population growth, urbanization, and industrialization (Jha et al., 2011). As a result, the implementation of a well-functioning waste management is one of the most important tasks of our modern societies.

Therefore, various strategies were developed to replace inefficient waste processing policies by such that are both economically feasible and ecologically sound.

In recent years the source-separate collection and utilization of organic and inorganic waste fractions has become a popular trend in Europe. In Austria, for instance, a source separation of the organic fraction – so called biowaste (BW) – and the inorganic fraction (residue waste) of municipal waste became mandatory in 1995. According to bmlfuw (2015) about 933,000 t BW were generated in Austria in the year 2014. The predominant amount of collected BW is still treated on composting sites due to traditional and infrastructural reasons. This process is sometimes associated with uncontrolled leachate and methane (CH_4) emissions and is a net energy consumer (Walker et al., 2009). The anaerobic digestion (AD) of biodegradable waste is the more promising and sustainable method, by generating biogas for energy use and a stabilized N-rich fertilizer for land application at the same time (Insam et al., 2015).

High solid-state anaerobic digestion (SS-AD) is already conducted in different reactor types, operating in batch, fed-batch or continuous mode. Discontinuous AD could be applied in (a) percolation fermenters, like the BIOFERM, BEKON or LOOCK systems, (b) impoundment fermenters, like the RATZKA system, where reactors are floated temporary with leachate and (c) pile fermenters, like the BAG BUDISSA system, without any leachate addition. For continuous high SS-AD, (a) percolation fermenters, like the ISKA or ATZ systems, (b) horizontal mechanically mixed plug flow fermenters, like the KOMPOGAS or LINDE systems, and (c) vertical fermentation, without internal mixing, for example the DRANCO system, are applied (Weiland, 2010; Li et al., 2010).

On the downside, anaerobic technology facilities require larger (long-term) investments and the overall process and operating is

* Corresponding author at: Institut für Mikrobiologie, Universität Innsbruck, Technikerstraße 25d, 6020 Innsbruck, Austria.
E-mail addresses: a.walter@uibk.ac.at (A. Walter), Maraike.Probst@uibk.ac.at (M. Probst), stephan.hinterberger@mueller-umwelttechnik.at (S. Hinterberger), mueller.sen@mueller-umwelttechnik.at (H. Müller), Heribert.Insam@uibk.ac.at (H. Insam).

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more complex (Mata-Alvarez et al., 2000). In fact, concepts combining achievements of technologies could bridge the gap between economic and ecological perspectives.

The MOBIGAS concept (Pöttinger Entsorgungstechnik, Grieskirchen, Austria) aims at complementing and enhancing already existing regional infrastructure, such as communal composting plants, with an easy-to-install and -operate box-type system. Three to ten fermenter containers of 58 m^3 are treating organic waste by batch high SS-AD to produce biogas. Generated biogas is stored and combusted in a cogeneration container unit directly on-site to produce electric and thermal energy. Fermented sludge (=seed) can be used as fertilizer or is treated on the composting site to obtain a stabilized product. Furthermore, through its modular concept, regional or seasonal fluctuations in requirement can be adjusted quickly and economically. A more detailed description and illustrations of the box-type container system are presented by Pöttinger (2015).

Studies investigating high SS-AD in lab-scale experiments are available (Cho et al., 1995; Guendouz et al., 2010; Li et al., 2010; Yan et al., 2014). However, there is a lack of information, on high SS-AD in pilot- or large-scale operation over prolonged periods. Of the lab-scale studies only few explicitly address the methanogenic community and their interactions with physicochemical parameters (Li et al., 2013, 2014; Yan et al., 2014).

The aim of the present study was to investigate changing fermenter conditions in a running pilot-scale high SS-AD MOBIGAS container system over a period of 12 batches. Temperature and gas yields were measured online and physicochemical characteristics were investigated offline. The methanogenic community was detected with the ANAEROCHIP microarray. Based on these results specific genera and species were subsequently quantified via quantitative PCR (qPCR). Data were evaluated statistically in order to identify interactions between abiotic and biotic characteristics.

2. Material and methods

2.1. Container operation and sampling

Three box-type containers (C1, C2, C3) in Schlüßlberg (48°13′10″N, 13°52′21″E, Austria) were run timely offset in batches B1 to B12 (Table 1).

Input material consisted of homogeneously mixed inoculum from the forgone batch and the substrate material BW, a composition of fresh kitchen waste and bulking material (scrub and grass cuttings, mature compost), described in Table 1. The 58 m^2 containers were filled using a small shovel loader. After filling, self-heating through aerobic preprocessing (first ventilation step) was induced, until temperatures of 50–65 °C were reached. Subsequently, containers were closed and mesophilic SS-AD was started for three weeks. Mesophilic conditions (Table 1) were stabilised by an installed floor heater. Additionally, a percolation system with two rebound nozzles (Pöttinger, 2015) was installed to sprinkle undiluted container leachate in different percolate volumes on demand. Table 1 presents information of percolate volumes and the number of percolations applied in single batches. To recirculate, leachate was collected at the container bottom and pumped into a storage tank to close the cycle. After three weeks, SS-AD containers were emptied. Samples were collected. Anaerobically digested seed was used as inoculum in the next batch. The inoculum to substrate ratio (I/S) and percolation intensity were changed frequently from B1 to B12 to test different operation conditions (Table 1).

To preserve the microbial community composition, seed samples were taken immediately in a depth of 15 cm at five evenly distributed spots of the container pile. Fresh percolation samples were taken simultaneously from the percolation storage tank. Subsequently, samples were frozen for storage until analysis. Three out of these five samples were randomly picked to measure physicochemical properties and do DNA extraction.

2.2. Online and offline measured process parameters

Biogas amount and composition were measured online with an AwiFLEX-analysis system (Awite GmbH, Langenbach, Germany). Reactor temperature was recorded online with a 3×pt-100-ATEX measuring probe (EMSR Eurotherm GmbH, Vienna, Austria). The following process parameters indicating stability and efficiency were measured for seed and percolate at the end of each batch: Total solids (TS), volatile solids (VS), pH, electrical conductivity (EC), total inorganic carbonate (TIC), and volatile fatty acid (VFA) concentrations. Ammonia nitrogen (NH_4-N) was measured for percolate, exclusively. Fermented seed was chopped with a Quad Blade CH580 (KENWOOD, Vienna, Austria) to receive homogeneous sample. Five grams of chopped material were dried at 105 °C for 24 h and weighed after cooling in a desiccator to determine TS. VS were calculated as loss of weight after igniting the oven-dried residue at 550 °C for 5 h in a muffle furnace.

Other chemical properties were measured in 1/10 dilutions with distilled water. NH_4-N was measured photometrically using the colorimetric tube test from Macherey-Nagel (Düren, Germany). NH_3-N (FAN) was calculated from NH_4-N concentrations according to the formula of Calli et al. (2005). EC and pH were analysed with the multiparameter-tester HI 98121 (HANNA instruments, Rhode Island, USA). VFA/TIC was measured by titrating 1/3 diluted samples with 0.05 M sulphuric acid in a Titrator DL50 (Mettler Toledo, Vienna, Austria). To determine acetate, propionate, valerate, isovalerate, butyrate, isobutyrate in a High Performance Liquid Chromatography (HPLC), samples were loaded on a Varian Metacarb 87 H-column and detected at wavelengths of 200 nm. The detection limit was 0.05 mM. Percolate samples were analysed in the same manner without preprocessing.

2.3. DNA extraction

DNA extraction of all seed and percolate samples was conducted in triplicates using the NucleoSpin®Soil extraction kit (Macherey-Nagel, Düren, Germany), according to the instructions provided by the manufacturer. Amounts of 0.5 g seed and 1.5 ml percolate were used as template for DNA extraction.

2.4. 16S rRNA gene amplification and ANAEROCHIP-microarray hybridisation

The methanogenic community composition of seed and percolate samples of B7, B8, and B9 was analysed with the ANAEROCHIP microarray. Archaeal 16S rRNA gene PCR amplifications were performed in a FlexCycler Thermal cycler (Analytikjena, Jena, Germany) in 25 µl volume, with each standard reaction mix containing a final concentration of 1 X MyTaq reaction buffer (Bioline Reagents Ltd., London, UK), 200 µM each dNTP, 0.8 µM of Cy5 labelled forward primer 109f, 0.2 µM of the reverse primer 934r, to which a PO^{4-} group was attached at the 5′ end (Grosskopf et al., 1998), MyTaq DNA polymerase (0.04 U μl^{-1}; Bioline Reagents, London, UK), and sterile water. In addition, 10 mM TMAC (tetramethylammonium chloride) and bovine serum albumin (BSA; 2 µg ml^{-1}) were included in the reactions to enhance specificity. One µl of extracted DNA was applied directly to the 24 µl of PCR reaction mix. After an initial denaturation for 5 min at 95 °C, 1 min at 80 °C, 1 min at 55 °C and 2 min at 72 °C, amplification reactions proceeded with 33 cycles at 95 °C for 1 min, 55 °C for 1 min and finally 72 °C for 2 min. Temperature cycling was

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