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Reactor performance of a 750 m^3 anaerobic digestion plant: Varied substrate input conditions impacting methanogenic community

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ABSTRACT

A 750 m^3 anaerobic digester was studied over a half year period including a shift from good reactor performance to a reduced one. Various abiotic parameters like volatile fatty acids (VFA) (formic-, acetic-, propionic-, (iso-)butyric-, (iso-)valeric-, lactic acid), total C, total N, NH_4 -N, and total proteins, as well as the organic matter content and dry mass were determined. In addition several process parameters such as temperature, pH, retention time and input of substrate and the concentrations of CH_4, H_2, CO_2 and H_2S within the reactor were monitored continuously. The present study aimed at the investigation of the abundance of acetogens and total cell numbers and the microbial methanogenic community as derived from PCR-dHPLC analysis in order to put it into context with the determined abiotic parameters. An influence of substrate quantity on the efficiency of the anaerobic digestion process was found as well as a shift from a hydrogenotrophic in times of good reactor performance towards an acetoclastic dominated methanogenic community in times of reduced reactor performance. After the change in substrate conditions it took the methano-archaeal community about 5–6 weeks to be affected but then changes occurred quickly.

1. Introduction

During the past decade anaerobic digestion (AD) to treat the organic fraction of wastes of different origins has increasingly gained importance for both, economic and ecological reasons. Biogas, the end-product of AD mainly consisting of CH_4 (60–70%) and CO_2 (30–40%), provides considerable potential as a versatile carrier of renewable energy, not solely because of the wide range of substrates that can be used for the AD process. Especially the combination of valuable gas production and simultaneous waste treatment including waste quantity reduction makes this biotechnology an essential part of future energy production, since gas obtained can either be used to generate electrical energy or as a fuel after purification [1].

Several process parameters are known to reflect the microbiological potential and activity during the AD process. Among others, the concentrations of volatile fatty acids (VFA) and ammonia, but also temperature, pH and hydraulic retention time are known to influence AD processes [2,3]. In previous studies [4,5] it was shown that the seasonality of the collected biowaste had a distinct influence on the efficiency of a digestion process. However, the causal interrelationships between abiotic parameters and the microorganisms are still a “black box” [6,7]. The present study investigated the reactor performance of a 750 m^3 anaerobic digestion plant extending over a period of half a year. During this period a shift from good reactor performance to a reduced one has been observed, taking the daily methane production as a basis. For this purpose various abiotic parameters were determined including the concentrations of volatile fatty acids (formic, acetic, propionic, (iso) butyric, (iso)valeric acid), concentrations of total C, total N, NH_4 -N, and organic matter content. Additionally, several process parameters such as temperature, pH, retention time, dry weight and input of substrate as well as the concentrations and amounts of CH_4, H_2, CO_2 and H_2S within the reactor were monitored continuously. The aim of the investigation was the evaluation of the abundance of acetogens, total cell numbers and the microbial methanogenic community as derived from PCR-dHPLC analysis in order to put these into context with the determined abiotic parameters.

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2. Material and methods

2.1. Biogas reactor and biowaste

A 750 m^3 thermophilic anaerobic digester (plug flow reactor) located in Roppen, Austria and running according to the KOMPOGAS-dry fermentation principle was investigated in the present study. This digestion plant is one of 4 large scale digesters in Austria processing a total of up to 10 000 t biological waste a^{-1}. For characteristic fermenter running conditions and some overall performance parameters please refer to [5,8,9].

2.2. Analysis

Amounts of biowaste and important process data like input and output of wastes and liquids, hydraulic retention times, temperatures at three different sampling points, and the quality and quantity of important gaseous components (CH_4, CO_2, H_2, H_2S) were analyzed automatically (gaseous components every two hours, other parameters continuously). In regular intervals of one week various additional parameters were determined in 3 replicates. For this purpose approx. 1 kg substrate was taken from the inlet sampling port of the digester. The pH was determined electrochemically. Total elemental concentrations of carbon and nitrogen (C_{tot}, N_{tot}) were determined using a CHNS-Analyzer (Leco TrueSpec) at 950 °C. NH_4—N and NO_3 -N were determined spectrophotometrically following the methods described by Schinner et al. [10]. Concentrations of several organic acids (formic -, acetic -, propionic -, butyric -, iso-buturic -, valeric -, iso-valeric -, lactic acid) were determined within the fermenter sludge following the method described in Wagner et al. [11] using a Shimadzu Prominence HPLC-system and an Aminex HPX87-H column. Protein concentration was determined using the Bradford method [12].

Preparation of the inoculum for the determination of acetogenic microorganisms was described previously [13]. Quantification of acetogenic microorganisms was carried out following a method from Harriot and Frazer [14]. The procedure was conducted under N_2 atmosphere in a glove-box (Mecaplex, Switzerland). After inoculation microplates were transferred into an anaerobic jar containing an oxygen-depleted and CO_2 enriched atmosphere (Merck Anaerocult A). Hydrogen was added via a septum port to reach a final concentration of 0.5% in the headspace, and the microplates were incubated at 52 °C. After 14 days of incubation they were analyzed according to their colorization. Most probable numbers (MPN) were calculated using the method described at the homepage of the US Food and Drug Administration [15].

2.3. DNA-extraction, PCR, quantitative real-time PCR, and dHPLC analysis

DNA was extracted from 500 µl liquid sample using the NucleoSpin Soil DNA extraction Kit (Macherey & Nagel). All extraction steps were carried out according to manufacturer's protocols including lysis buffer SL1 and enhancer. DNA was eluted in 50 µl of elution buffer, reserve-aliquots were stored at −20 °C, working stocks at 4 °C. DNA concentration was measured spectrophotometrically using a NanoDrop 2000c (Thermo Scientific).

PCR with methanogen specific primers (O357fGC/O691r) [16] was carried out according to standard protocols as a nested PCR following 30 amplification cycles with general archaeal primers (arc109f/arc1059r) [17,18]. The reaction mixture contained 25 µl MyTaq™ 2 × Mix PCR mixture, primers in a final concentration of 0.25 µM, 50 µg bovine serum albumin (aqueous solution, filter sterilized), and PCR grade water to achieve a final volume of 50 µl. PCR-programs were used as described in Wagner et al. [13]. PCR products were loaded onto dHPLC after purification (PCR product clean-up, Macherey & Nagel).

Quantitative real-time PCR (qPCR) was used in order to determine the copy numbers of methanogenic microorganisms. For quantification of total methanogens a qPCR with the primer pair O357f/O691r [16] was applied, and a SensiFAST SYBR No-ROX kit (Bioline) was used for detection. The primer concentration was set to 0.2 µmol L^{-1} per reaction, 2 µl of DNA template were added to 18 µl of the final qPCR mix including 0.8 µl 5× TaqMaster PCR Enhancer (VWR, Germany) and the amplification was run for 40 cycles with the following conditions: 15 s at 95 °C, 20 s at 49 °C and 15 s at 72 °C. The samples were measured using a Corbett Life Science Rotor-Gene 6000 system (Qiagen), and after quantification PCR products were checked with melt curve analysis. dHPLC was carried out as described in Wagner et al. [19] using elution gradient parameters described previously [13]. To obtain additional information on the microbial methanogenic community pure culture amplicons of various methanogenic archaea (*Methanothermobacter wolfei*, *Methanothermobacter thermoautotrophicus*, *Methanosarcina thermophila*, *Methanosarcina acetivorans*, *Methanosarcina barkeri*, *Methanoculleus bourgensis*) were used in order to match peaks with the same retention time.

2.4. Statistical analysis

Statistical analysis was performed using the Software package Statistica 8 (StatSoft Inc.).

3. Results and discussion

3.1. Reactor performance and (abiotic) process parameters

During the half-year investigation period the reactor performance could be divided into different phases according to the daily methane production. The first phase (Phase A) from February 2009 until 19th May 2009 was characterized by a high methane production with an average of 2584 m^3 CH_4 day^{-1} (± 249), followed by a phase (Phase B) of reduced performance with significantly lower average methane production of 1900 m^3 CH4 day^{-1} (± 121) from 26th May until 28th July and again a phase (Phase C) of high methane production until Oct 2009 with 2452 m^3 CH4 day^{-1} (± 156) (Table 1). This decreased reactor performance was attributed to a reduced substrate input. This supports previous findings of [4,5]. The relation of daily methane production and substrate input is depicted in Fig. 1. Although the reduced input of organic material is attributed to changes in the quantities in available biowaste, biogas plant operators can hardly omit this problem. During the touristic season in Tyrol, Austria, next to the quantity of biowaste also its composition is changing. However, an impact of the changes in substrate was reflected neither by the total carbon content (C_{tot}), the total nitrogen content (N_{tot}) or the C/N ratio nor by the total concentration of proteins. Moreover, statistical analysis of VFAs (formate, acetate, propionate, (iso-)butyrate, (iso-)valeric, lactic acid) data including the actate:propionate ratio and total VFAs, ammonia concentration, organic matter content, buffer capacity, pH, and electrical conductivity did not reflect the phases in an appropriate manner, although (significant) differences between them were found. A summary of those data obtained for the 3 phases is depicted in Table 1.

However, the reduced reactor performance could only be described quantitatively by the overall substrate input but not qualitatively by the resulting change in chemical properties. Therefore various microbial parameters were determined to see if the established microbial community is affected by or are the cause for the changes described above.