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Effect of moisture of municipal biowaste on start-up and efficiency of mesophilic and thermophilic dry anaerobic digestion

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HIGHLIGHTS

- Dry anaerobic digestion needs ⩾75% moisture.
- *Methanosarcinales* dominate, no *Methanosaeta* spec.
- Biogas/methane rates and amounts are equal at 37 and 55 °C.

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ABSTRACT

Methane production from biowaste with 20–30% dry matter (DM) by box-type dry anaerobic digestion and contributing bacteria were determined for incubation at 20, 37 and 55 °C. The same digestion efficiency as for wet anaerobic digestion of biowaste was obtained for dry anaerobic digestion with 20% DM content at 20, 37 and 55 °C and with 25% DM content at 37 and 55 °C. No or only little methane was produced in dry anaerobic reactors with 30% DM at 20, 37 or 55 °C.

Population densities in the 20–30% DM-containing biowaste reactors were similar although in mesophilic and thermophilic biowaste reactors with 30% DM content significantly less but phylogenetically more diverse archaea existed. Biogas production in the 20% and 25% DM assays was catalyzed by *Methanosarcinales* and *Methanomicrobiales*. In all assays *Pelotomaculum* and *Syntrophobacter* species were dominant propionate degraders.

1. Introduction

Biowaste is the moist organic fraction of municipal garbage with a high percentage of organic matter and is collected separately in many municipalities of Germany. After separation of non-digestible material it can be treated by wet or dry anaerobic digestion (e.g., Lissens et al., 2001; Luning et al., 2003; de Baere, 2000; Nayono et al., 2009). Reports on dry anaerobic digestion (DAD) were dealing with stirred tank reactors and biowaste fractions that contained up to 25% dry matter (DM) (e.g., Cecchi et al., 1991; Mata-Alvarez et al., 1993; Pavan et al., 2000; Bolzonella et al., 2006). Only Abbassi-Guendouz et al. (2012) reported successful DAD of artificial biowaste (card boards) with a DM content of 30% in 2 of 4 parallel reactors. Model equations for batch fermentation revealed that mass transfer was strongly limited in the DAD reactors with ⩾30% DM content, leading to local acidification and reduced methanogenic activity (Abbassi-Guendouz et al., 2012). Even if an almost complete hydrolysis of biowaste solids could be obtained by thermochemical or biological pretreatment to increase the soluble COD (Fernandez-Güelfo et al., 2011) the synergistic action of fermentative bacteria with methanogens and that of acetogenic bacteria with acetate cleaving and hydrogenolytic methanogenic bacteria was still required for stable methane production (Gallert and Winter, 2005).

Although in praxi DAD often is established in low-tech box fermenters ("garage fermenters") and in high-tech stirred tank vertical or horizontal reactors the focus of most literature reports was layed on stirred tank reactors with much better mass transfer properties. Almost no information about methanogenesis in box fermenters is available. Box fermenters for DAD are considered inexpensive and meet the requirements for the "technology bonus" (2 Euro cent per kWh electricity) offered by the German Renewable Energy Law. No process water is added to DAD during reactor feeding, but in some systems process water or leachate is sprayed on or mixed into the digesting material to improve biogas

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production and digestion efficiency. Anaerobic microbial consortia for biogas production from organic matter require an aequous environment with a water activity of >0.91 (e.g., Rockland and Beuchal, 1987) for high-rate hydrolysis of polymers, acidogenesis of monomers, acetogenesis of fatty acids and methanogenesis of acetate and of CO_2/H_2. At the high dry matter (DM) content of non-moistened municipal biowaste ($\geqslant$30%) there may not be enough bioavailable water ($a_W \leqslant 0.91$) for an optimal, non water limited multistep DAD. As waste treatment by DAD began only about 20 years ago, not many data on the behavior of DAD during start-up and during long-term fermentation are available. Abbassi-Guendouz et al. (2012) reported a slightly decreasing methane production from card boards for increasing DM content from 10% to 25%. At 30% DM content methanogenic activity was no longer stable and at 35% DM content methanogenesis failed completely. The authors concluded that 30% DM content was the threshold concentration for the solids content. Simulation with the anaerobic digestion model No.1 revealed mass transfer limitations at increasing DM content and in particular a limited hydrolysis rate at high solids content. In another report mesophilic and thermophilic DAD of the organic fraction of municipal solid waste (OFMSW with 20% DM) was compared by modeling organic matter conversion and biogas production (Fernàndez-Rodriguez et al., 2013). Specific growth rates were 27–60% higher during thermophilic than during mesophilic methanogenesis, which could have been due to an increased water activity. More water of the moisture was apparently bioavailable at 55 °C than at 37 °C. As a consequence thermophilic reactor operation would require a shorter retention time and presumably less investment costs for the digester.

The start-up of reactors for wet anaerobic digestion (WAD) or DAD depends on the activity of the inoculum, which ideally should come from digestion of the same or of a similar substrate. A major problem during the start-up phase may be the accumulation of volatile fatty acids, especially of propionate by the fast-growing heterotrophs (Gallert and Winter, 2008; Felchner-Zwirello et al., 2012, 2013), which lead to acidification of the reactor content and, if no counteractions are taken, to failure. Monitoring of fatty acids for flexible biowaste addition may shorten the start-up time and is considered helpful for a successful start-up of bioreactors, e.g., after revision (Gallert et al., 2003; Gallert and Winter, 2008). High-rate anaerobic digestion by WAD or DAD depends on syntrophic interaction of fatty acid degrading acetogens with acetate and H_2/CO_2 utilizing methanogens to avoid volatile fatty acid accumulation (Gallert and Winter, 2005). If fatty acids have been formed due to process imbalances, propionic acid is the most critical organic acid, since its degradation may depend on the established degradation pathway (Felchner-Zwirello et al., 2012), the hydrogen partial pressure and on acetate levels. High degradation rates require close interspecies distances between propionate degraders, hydrogenolytic and aceticlastic methanogens (Felchner-Zwirello et al., 2013) that prevail during DAD, but also a non-limiting water activity as in WAD.

Little is known about the community structure in DAD reactors. Within the methanogens a dominance of *Methanosarcinales*, either of *Methanosaeta* spec. (Chu et al., 2010; Montero et al., 2009) or *Methanosarcina* spec. (Cho et al., 2013) was reported, whereas propionate degraders, with one exception (Zahedi et al., 2013), were only investigated in WAD systems (e.g., Ariesyady et al., 2007; Felchner-Zwirello et al., 2012; Narihiro et al., 2012; Chu et al., 2010).

In this contribution we address three aspects of DAD: the optimal water content, the influence of a temperature change from ambient (20 °C) to mesophilic (37 °C) and thermophilic (55 °C) temperatures as well as identification and enumeration of dominant methanogens and propionic acid degraders with respective gene probes.

2. Methods

2.1. Substrates: source of fresh biowaste and digester residues for inoculation

Source-sorted biowaste was collected with rotating drum trucks by City authorities of Karlsruhe, Germany for large-scale wet anaerobic digestion (WAD) (Gallert et al., 2003; Nayono et al., 2009). For lab-scale dry anaerobic digestion (DAD) experiments woody material, ornamental plant soils as well as paper, plastic foils, broken glass and metals were manually sorted out from the collected biowaste fraction before shredding in a cutter (ZG Raiffeisen, Karlsruhe) to 1 cm length. The dry matter (DM) content of the sorted biowaste (triplicate analyses) was 30.9 ± 0.6% (first batch for start of experiments) and 30.3 ± 0.6% (second batch for the re-feeding experiments). In both batches the organic dry matter content (ODM, triplicate analyses) varied only little between 65% and 67% of the DM content (calculated by ODM/DM× 100, Table 1). As a source of microorganisms solid residues of digested biowaste suspensions were taken from the extrusion pipe of the sludge centrifuge at the WAD plant of the City of Karlsruhe. This inoculum contained 33.7 ± 0.6% DM of which 62% were organic material (bacteria and undigested/undigestible biowaste particles). Portions of 10 kg of shredded fresh biowaste and of 10 kg solid residues of digested biowaste suspension were mixed thoroughly. Little water was added to obtain a calculated DM content of 30%, which was controlled in each fermenter by respective analyses. To obtain biowaste fractions with 25% or 20% DM content (Table 1), the above mixture was accordingly diluted with tap water. Percent amounts were calculated from the mean of triplicate analyses (variation ±0.6%). After 300 days all DAD reactors were re-fed (Table 2).

2.2. Digester set-up, feeding and incubation conditions

Parallel DAD experiments with 2 kg of the above prepared biowaste fractions that contained 30%, 25% or 20% DM were started in 3 L glass reactors. One reactor was only fed with 2 kg digester residues that contained 25% DM and was incubated as a control. Reactors were initially flushed with nitrogen, closed with a rubber stopper and incubated at room temperature (20 ± 1 °C). Incubation time, changes of the incubation temperature, e.g., after re-feeding, pH corrections with 5 M NaOH are mentioned at the respective experiments. Biogas from the 3 L reactors was measured with a Ritter model MGC-1 V30 mini gas counter, analyzed with a Blue sense model BACCom 12 CB methane/CO_2 gas detector and registered by a computer (System Blue Sense Gas GmbH, D-45099 Herten, Germany). Since no gas was produced after 3 months of incubation in the control reactor, it was stopped. Little leachate water that accumulated at the bottom of the reactors after 3–4 days was regularly remixed into the solid fraction by shaking the reactors. For measuring the pH and for analysis of volatile fatty acids 1 ml of leachate was withdrawn through a bottom valve. Initial incubation of all reactors was at room temperature (20 ± 1 °C). The pH was adjusted after 5, 10 and 30 days to 8 (as good as this is possible at the high DM content) and then stabilized itself at around 8 (Figs. 1b, 2b, 3b and c). Later on the incubation temperature was raised to 37 ± 0.5 or 55 ± 0.5 °C as indicated in Figs. 1–3. Re-fed reactors were incubated at 37 ± 0.5 or 55 ± 0.5 °C.

2.3. Analyses

To determine the DM content of biowaste three 1-kg portions were dried to constant weight at 105 °C. The organic DM (ODM) content was obtained from triplicate 20 g-portions of the united