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Research note

Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste

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Abstract

Changes in methanogenic population levels were followed during startup of a full-scale, farm-based anaerobic sequencing batch reactor (ASBR) and these changes were linked to operational and performance data. The ASBR was inoculated with anaerobic digester sludge from a municipal wastewater treatment facility. During an acclimation period of approximately 3 months, the ASBR content was diluted to maintain a total ammonia-N level of approximately 2000 mg l^{-1}. After this acclimation period, the volatile solids loading rate was increased to its design value of 1.7 g l^{-1} day^{-1} with a 15-day hydraulic retention time, which increased the total ammonia-N level in the ASBR to approximately 3600 mg l^{-1}. The 16S ribosomal RNA (rRNA) levels of the acetate-utilizing methanogens of the genus *Methanosarcina* decreased from 3.8% to 1.2% (expressed as a percentage of the total 16S rRNA levels) during this period, while the 16S rRNA levels of *Methanosaeta concilii* remained low (below 2.2%). Methane production and reactor performance were not affected as the 16S rRNA levels of the hydrogen-utilizing methanogens of the order *Methanomicrobiales* increased from 2.3% to 7.0%. Hence, it is likely that during operation with high ammonia levels, the major route of methane production is through a syntrophic relationship between acetate-oxidizing bacteria and hydrogen-utilizing methanogens. Anaerobic digestion at total ammonia-N levels exceeding 3500 mg l^{-1} was sustainable apparently due to the acclimation of hydrogen-utilizing methanogens to high ammonia levels. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Ammonia; Anaerobic; Anaerobic sequencing batch reactor; Methanogens; Swine waste; 16S rRNA

1. Introduction

Anaerobic treatment of animal waste is an attractive treatment strategy because a large quantity of methane (and thus energy) can be produced due to the high organic content of animal wastes. Other advantages of anaerobic treatment of animal waste include: (i) conservation of nutrients, (ii) greater than 50% solids destruction, and (iii) reduction of odor. However, post-treatment of anaerobic effluent may be necessary if land application or discharge of effluent requires low nutrient levels.

Most full-scale animal waste treatment systems consist of traditional anaerobic digesters, such as covered anaerobic lagoons and completely stirred tank reactors (CSTR). A typical volatile solids loading rate (VSLR; grams of volatile solids [VS] fed per liter reactor

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volume per day) for a non-heated covered anaerobic lagoon is 0.24 g l^{-1} day^{-1} with a hydraulic retention time (HRT) of 65 days [1]. Design parameters for a CSTR are dependent on the VS concentration in the influent and operating temperatures. Using a conventional retention time for a mesophilic (35°C) anaerobic CSTR of 15 days and assuming that swine waste is introduced with a concentration of 20 g VS l^{-1}, the resulting VSLR for such a system is 1.3 g l^{-1} day^{-1}. During the past 30 years, high-rate anaerobic treatment systems (characterized by a high ratio of solids retention time [SRT] over HRT) have become popular, because of the smaller reactor volume, and hence lower construction costs. However, so far, high-rate systems mostly have been operated for the treatment of low-solids wastewaters [2]. Recent developments such as the anaerobic sequencing batch reactor (ASBR) and the anaerobic baffled reactor have made it possible to treat high-solids waste streams (e.g., animal waste) by high-rate systems as well [3–5]. For example, we demonstrated successful treatment (59% VS reduction) of swine waste with a VS concentration of 20 g l^{-1} at a VSLR of up to 5.7 g l^{-1} day^{-1} and a 3.3-day HRT using lab-scale ASBR systems operated at 25°C [6]. To further demonstrate the feasibility of the ASBR to treat swine waste, a full-scale ASBR system was operated at a farm. We followed changes in methanogenic population levels during startup of this farm-based digester and linked these population data to operational and performance data.

2. Materials and methods

2.1. Full-scale ASBR

A circular 600-m^3 ASBR (diameter of 15 m and a wet-volume height of 3.4 m) was built on a 3000-head (average weight of 60 kg) feeder-to-finish swine farm in Nevada, IA, USA (Fig. 1). Construction was completed in the fall of 1998. The design VSLR of the reactor was

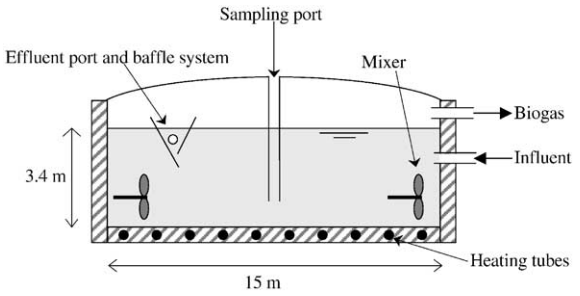

Fig. 1. Schematic of full-scale, circular ASBR. The effluent port and baffle system and the heating tubes are perpendicular to the page.

intended to be 4 g l^{-1} day^{-1}, but due to an overestimation of the anticipated loading rate, the reactor was over-designed by a factor of 2.4. The actual VSLR was calculated to be approximately 1.7 g l^{-1} day^{-1} with a 15-day HRT according to tables in the Agricultural Waste Management Field Handbook [7]. The reactor was constructed with concrete panels embedded in soil and a concrete floor in which a heating-tube system was placed. An insulated, inflatable cover was installed so that the headspace of the system also could be used for biogas storage. A boiler that burned biogas or propane was utilized to heat the reactor contents to approximately 25°C.

The cycle length of the ASBR was 8 h and consisted of a feed step (0.2 h), a react step (6.8 h), a settling step (0.5 h), and a decant step (0.5 h). The ASBR was fed three times per day with swine waste from one of the three 1000-head confinement buildings (scrape and flush systems). It took 2 days to empty one building, and hence it took 6 days to empty all three buildings. Intermittent mixing (every hour for 15 min) during the feed and react steps was provided by two 4-hp mixers (model 4640/316L, ITT Flygt, Trumbull, CT, USA). The settling step introduced partial separation between liquid and solids, while effluent was decanted by gravity via an effluent baffle system, which provided additional solids/liquid separation. The effluent was introduced in a settling tank to accomplish additional solids removal, and subsequently was transferred to an aerated polishing tank with a retention time of 30 min. Half of the liquid flow from this polishing tank was recycled to the confinement buildings as flush water. The other half was stored in a lagoon and applied to agricultural land during the growing season.

The reactor was seeded with 50 m^3 secondary anaerobic digester sludge (30 g VS l^{-1}) from the municipal wastewater treatment plant of the City of Ames, IA, USA in October 1998. The reactor was partly filled with swine waste (10 m^3) and groundwater (200 m^3) one week before adding secondary digester sludge. Due to problems with the heating system, startup of the reactor did not proceed until the middle of December 1998. During an acclimation period, groundwater was used to dilute the reactor contents from February 1 to April 29, 1999. Finally, on April 30, 1999, the VSLR was increased to the design loading rate of approximately 1.7 g VS l^{-1} day^{-1}.

2.2. Laboratory-scale ASBR

A 84-l ASBR (inside dimensions: length 0.30 m, width 0.30 m, height 1.22 m, and wet-volume height 0.91 m) was built from 1.3-cm-thick Plexiglas. A baffle effluent system was placed before the effluent port [8]. The system was operated at room temperature (approximately 22°C) at a VSLR of approximately 4 g

$VS\,l^{-1}\,day^{-1}$. Cycle length and intermittent mixing duration were similar to those of the full-scale ASBR, except that feeding and decanting steps were slightly shorter. Mixing was performed by recirculating biogas through a diffuser system that was placed in the bottom of the reactor. The biogas collection system consisted of a gas bag to prevent a pressure drop in the headspace during decanting of effluent, an observation bottle, and a wet-tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA). Programmable timers (ChronTrol Corporation, San Diego, CA, USA) were used to control reactor operation. All pumps were Masterflex pumps of Cole Parmer Instrument Co. (Chicago, IL, USA).

The reactor was seeded with 251 primary digester sludge from the municipal wastewater treatment plant of the City of Ames, IA, USA. Swine waste was obtained once a week from the swine farm where the full-scale ASBR was operated. The waste was screened using a 2.36-mm sieve to prevent the reactor ports from clogging with large debris. Since the screened swine waste was not diluted with water, the concentration of swine waste fluctuated between 30 and $100\,g\,l^{-1}$ depending on farm operation.

2.3. Analyses

Total solids, VS, and total volatile fatty acid (VFA) concentrations were determined according to procedures in *Standard Methods* [9]. Total ammonia (ammonium and ammonia) concentrations were measured by raising the pH to 11 to convert all ammonium to ammonia and subsequently measuring the ammonia concentration using an ATI Orion Model 720A Benchtop pH/ISE meter and an ammonia probe (ATI Orion, Boston, MA). The composition of the biogas was measured using gas chromatography (Model 350 Gow-Mac Instruments Co., Bridgewater, New Jersey, USA) with thermal conductivity detector (column: $1.7\,m \times 3\,mm$ stainless steel Poropack Q 80/100 mesh). The biogas production was measured with a thermal mass flow meter (Fluid Components International Co., San Marcos, CA, USA).

2.4. Hybridizations

Biomass samples were taken (after a mixing event) from a sampling port on top of the reactor (Fig. 1) by using a peristaltic pump and flexible 1.3-cm diameter tubing. Samples were transported to the laboratory on wet ice and centrifuged at $350g$ at 4°C. Subsequently, the supernatant was removed and the biomass pellet was frozen rapidly in a mixture of ethanol and dry ice and stored at −80°C. For quantitative membrane hybridizations, RNA was extracted from these samples by a low-pH hot-phenol extraction method, denatured, applied to Magna Charge membranes (Micron Separation Inc., Westborough, MA, USA), and hybridized with [γ-^{32}P] ATP-labeled oligonucleotide hybridization probes [10]. The hybridization signal was quantified using an Instant Imager (Packard Instruments Company, Meriden, CT, USA) and the abundance of each phylogenetic target group was expressed as a percentage of the total 16S ribosomal RNA (rRNA) determined by using a universal probe (S-*-Univ-1390-a-A-18) [10,11] (Table 1). Standard deviations were evaluated by error

Table 1
Oligonucleotide probes used, their target groups, relevant characteristics, and references

Probe[a]	Target group	Relevant characteristics[b]	Reference
S-*-Univ-1390-a-A-18	Virtually all organisms		[11]
S-D-Bact-0338-a-A-18	Virtually all *Bacteria*		[12]
S-D-Arch-0915-a-A-20	Virtually all *Archaea*		[13]
S-O-Mmic-1200-a-A-21	*Methanomicrobiales*	Most use H_2-CO_2 and formate	[14]
S-F-Mbac-0310-a-A-22	*Methanobacteriaceae*	Most use H_2-CO_2, some use H_2-CO_2 and formate	[14]
S-F-Mcoc-1109-a-A-20	*Methanococcaceae*	Most use H_2-CO_2 and formate	[14]
S-G-Msar-0821-a-A-24	*Methanosarcina* spp.	Use acetate and other substrates (H_2-CO_2, methanol, and methylamines), exhibit high half saturation constants and maximum specific growth rates for acetate	[14]
S-S-M.con-0381-a-A-22	*M. concilii*	Use only acetate, exhibit low half saturation constants and maximum specific growth rates for acetate	[15]

[a] Probe nomenclature according to Alm et al. [16].
[b] According to Raskin et al. [17].

propagation from triplicate applications of the same rRNA sample to the membrane, and hence do not represent a real triplicate of independent biomass samples obtained from the reactor.

3. Results and discussion

The methanogen probes used in this study (Table 1) target most methanogens believed to be relevant in anaerobic bioreactors [15,18,19]. These methanogens belong to four of the five described orders of methanogens, i.e., *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales*, and *Methanosarcinales*. Representatives of the fifth order of methanogens, *Methanopyrales*, are extremely thermophilic [33] and are not likely to present in anaerobic bioreactors. Therefore a probe was not used to target methanogens within this fifth order.

The abundance of methanogenic populations in biomass samples collected from the two primary and the secondary anaerobic digesters of the municipal wastewater treatment plant of the City of Ames, IA, USA are shown in Fig. 2a. Relative 16S rRNA levels of the acetate-utilizing methanogen *Methanosaeta concilii* and hydrogen-utilizing methanogens of the order *Methanomicrobiales* were higher in the secondary digester (14.4% and 10.6%, respectively) compared to the levels in the primary digesters (10.1% [east], 7.7% [west] and 9.4% [east], 7.1% [west], respectively). Acetate-utilizing methanogens of the genus *Methanosarcina* were less abundant in the digester samples (Fig. 2a), which suggests that the acetate concentrations in the anaerobic digesters of the wastewater treatment plant were low (see below). Hydrogen-utilizing methanogens from the families *Methanococcaceae* and *Methanobacteriaceae* were relatively low in all digester samples (Fig. 2a).

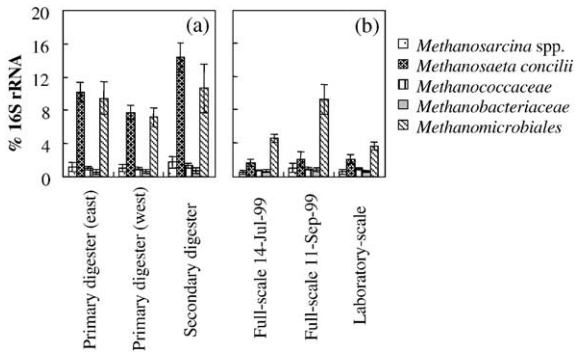

Fig. 2. Relative hybridization signal of methanogen probes: (a) wastewater treatment plant anaerobic digester samples and (b) samples from full-scale and laboratory-scale ASBRs treating swine waste from the same farm.

Based on the higher levels of methanogens in the secondary digester, we decided to use sludge from this digester as the inoculum for the full-scale ASBR. The reactor was inoculated in the middle of October 1998. Due to problems with the heating system, the reactor was not fed until the middle of December 1998. Fig. 3a

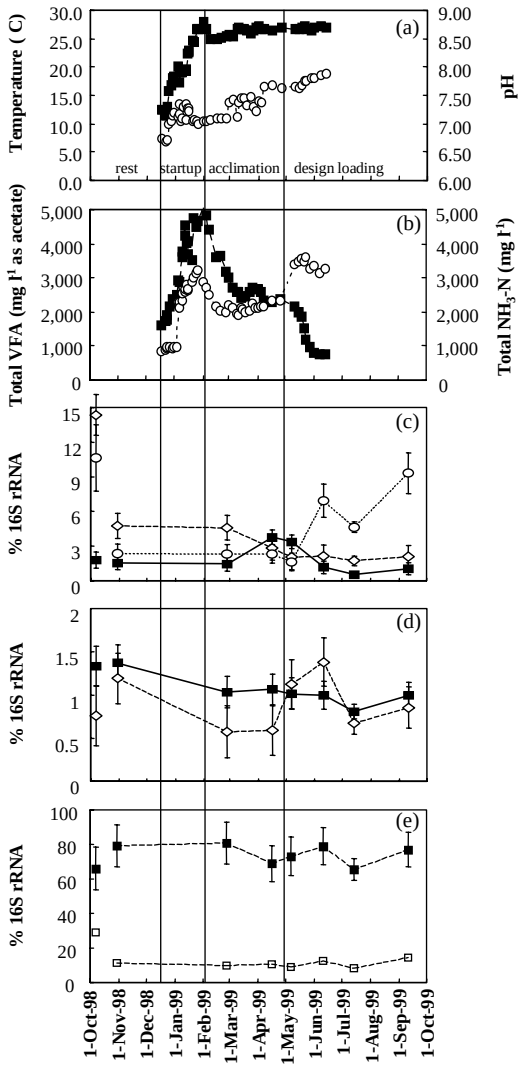

Fig. 3. Reactor operating data, performance data, and relative hybridization signals of various probes versus operating time: (a) temperature (■) and pH (○); (b) total VFA (■) and ammonia-N concentration (○); (c) *Methanomicrobiales* (○), *Methanosarcina* spp. (■), and *M. concilii* (◇) 16S rRNA levels; (d) *Methanobacteriaceae* (■) and *Methanococcaceae* (◇) 16S rRNA levels; and (e) bacterial 16S rRNA levels (■) and sum of hybridization signals obtained with individual methanogen probes (□). Data points for the beginning of October 1998 are for samples obtained from the secondary anaerobic digester (see Fig. 2a).

shows the temperature and pH of the ASBR during operation. The design temperature of 25°C was reached by the end of January 1999. Meanwhile, total VFA and ammonia-N concentrations rose to approximately 5000 and 3200 mg l^{-1}, respectively, by the end of the startup period (Fig. 3b), which indicated that hydrolytic and fermenting bacteria were active. Despite high total VFA levels, the pH remained favorable for methanogenesis (Fig. 3a), because of the buffering capacity of the high levels of ammonia produced in the system.

Increasing levels of total VFAs during January 1999 showed that the abundance and/or activity of acetogens and methanogens were insufficient to convert the VFAs produced. Improvement in performance through degradation of VFAs was not anticipated, because the high total VFA and ammonia levels were likely inhibitory for acetogens and methanogens. Therefore, starting on February 1, 1999, groundwater was used to dilute the reactor contents until total VFA and ammonia-N levels were approximately 2500 and 2000 mg l^{-1}, respectively (Fig. 3b). As a result of this dilution, the VSLR was reduced. When methane production reached a level of 800 m^3 day^{-1} (biogas consisted of $74 \pm 0.5\%$ methane) by the end of April 1999, the VSLR was increased to the design loading rate of approximately 1.7 g VS l^{-1} day^{-1}. As a result, the total ammonia-N level increased to approximately 3600 mg l^{-1}. Meanwhile, total VFA concentrations decreased to approximately 750 mg l^{-1} by the middle of June 1999.

Figs. 3c and d show relative 16S rRNA levels of five groups of acetate-utilizing and hydrogen-utilizing methanogens over the operating period. As discussed above, it was demonstrated in previous studies that the combined use of these probes targets most or all methanogens in a variety of mesophilic, anaerobic bioreactors [15,18,19]. A summation of the relative 16S rRNA levels of these five groups of methanogens (corresponding to the total 16S rRNA level of organisms of the domain *Archaea*) and the relative 16S rRNA levels of organisms of the domain *Bacteria* showed that, on average, 88.4% of the total 16S rRNA was detected by the probes used in this study (Fig. 3e). A lower than 100% coverage was anticipated since no probe was used to determine the levels of organisms of the domain *Eucarya*.

After addition of the inoculum to the mixture of groundwater and swine waste and before the startup period (i.e., from October 15 to December 15, 1998), the levels of the main methanogenic populations (*M. concilii* and *Methanomicrobiales*) decreased substantially, whereas the levels of other methanogens remained constant (*Methanosarcina* spp. and *Methanococcaceae*) or increased (*Methanobacteriaceae*). Methanogenic populations remained at similar levels or declined during the startup period and the first month of the acclimation period, during which methanogenic activity was low (indicated by high levels of total VFA and ammonia and virtually no methane production). After dilution and the reduction in VSLR on February 1, 1999, and the subsequent decrease in total VFA and ammonia-N levels to approximately 2500 and 2000 mg l^{-1}, respectively, relative levels of *Methanosarcina* spp. increased from 1.4% to 3.8%. This increase coincided with a small decrease in total VFA concentration from 2679 to 2162 mg l^{-1} in April 1999 (Fig. 3b) and an increase in methane production. The competitive advantage of *Methanosarcina* spp. as the main acetate-utilizing methanogen was not surprising given the high total VFA concentration of approximately 2500 mg l^{-1} (which presumably corresponded to a high acetate concentration). It is generally accepted that the generalist *Methanosarcina* (with a high growth rate) is more competitive in systems with a high acetate concentration, while the specialist *M. concilii* (with a high affinity for acetate) is selected in systems with a low acetate concentration [20,21]. In addition, Sprott and Patel [22] found that methane formation from *M. concilii* was completely inhibited at a total ammonia-N level of 560 mg l^{-1}, while methane formation from *Methanosarcina barkeri* was not inhibited at a total ammonia-N level of 2800 mg l^{-1}. Hence, the combination of high acetate levels (as suggested by high VFA concentrations) and total ammonia-N levels exceeding 2000 mg l^{-1} may have selected for *Methanosarcina* spp. as the abundant acetate-utilizing methanogens.

After the end of the acclimation period when the VSLR was increased to the design VSLR of 1.7 g l^{-1} day^{-1}, the total ammonia-N levels increased rapidly from 2330 to 3580 mg l^{-1}. Subsequently, relative signals of *Methanosarcina* spp. again decreased from 3.8% to 1.2% and *Methanomicrobiales* increased from 2.3% to 7.0%. We assume that *Methanosarcina* spp. were inhibited by the very high total ammonia levels in the reactor. This phenomenon was also observed under thermophilic conditions by Angelidaki and Ahring [23], who postulated that acetate-utilizing methanogens were more sensitive to sudden increases in the total ammonia concentration than hydrogen-utilizing methanogens. These observations are in agreement with other reports for mesophilic conditions [22,24–26]. Moreover, Koster and Lettinga [27] reported that above a threshold total ammonia-N level of 1700 mg l^{-1} acetate-utilizing methanogens (under mesophilic conditions) were more sensitive to increases in the total ammonia concentration than hydrogen-utilizing methanogens, while the opposite was observed for total ammonia levels below this threshold. The less sensitive character of hydrogen-utilizing methanogens for high total ammonia concentrations apparently saved our reactor from failure, as the levels of the hydrogen-utilizing methanogens from the order *Methanomicrobiales* increased after the levels of *Methanosarcina* spp. had decreased. Methane

production during this period remained favorable and total VFA levels decreased to 750 mg l^{-1}.

It should be noted that, in contrast to our findings and the information in the literature described above, other studies reported that acetate-utilizing methanogens were able to acclimate to total ammonia-N levels up to 4000 mg l^{-1} for mesophilic and thermophilic conditions [28,29].

After the increase in the concentration of total ammonia, methane production apparently was mostly channeled through hydrogen as an intermediate. Our observations of increased acetate degradation and low *M. concilii* and *Methanosarcina* spp. levels are consistent with other studies that have suggested that in some anaerobic systems a syntrophic relationship between an acetate-oxidizing organism (possibly a homoacetogen) and a hydrogen-utilizing methanogen serves as the major route of methane production from acetate [30]. The production of methane from acetate through this syntrophic consortium appears to be more common in stressed systems including systems characterized with high salt and total ammonia levels [26,31,32]. Most hydrogen-utilizing methanogens remained at low levels during the entire operating period. A substantial increase in the relative levels of the order of *Methanomicrobiales* and a less significant increase in the levels of the family of *Methanobacteriaceae* during the first part of May, 1999 coincided with a decrease in *Methanosarcina* spp. This may support the hypothesis of a syntrophic relationship between acetate oxidizers and hydrogenotrophic methanogens, which explains the continuation of methane production during a sudden increase in the total ammonia level by an increase in hydrogen-utilizing activity.

At the end of the 1-year sampling period, the methanogenic community structure did not yet appear to be stable in the full-scale ASBR (Fig. 3c and d). A laboratory-scale ASBR that was fed with the same swine waste for 1.5 years (with a total ammonia-N concentration in the reactor of approximately 4100 mg l^{-1}) showed a methanogenic community structure similar to the one in the last two samples obtained from the full-scale digester, but with a slightly lower level of *Methanomicrobiales* (Fig. 2b). Hence, it is likely that, even after a period with high total ammonia levels longer than the acclimation period in this full-scale ASBR study, methane will be produced primarily by hydrogen-utilizing methanogens.

4. Conclusions

We demonstrated that it was feasible to operate a full-scale, on-farm ASBR system for the treatment of swine waste. Careful monitoring of total ammonia and total VFA levels and flexibility in operation during startup were essential to successfully operate this anaerobic digester. For example, after an initial startup period, the reactor contents were diluted with groundwater to reduce the high total ammonia-N levels from about 3200 mg l^{-1} to approximately 2000 mg l^{-1}. As a result, reactor performance improved and operation at the design VSLR of 1.7 g VS l^{-1} day^{-1} was initiated. At this design VSLR, the total ammonia-N levels increased again to 3600 mg l^{-1}, but this increase did not affect biogas production. However, the relative levels of acetate-utilizing methanogens of the genus *Methanosarcina* decreased, while levels of acetate-utilizing methanogens of the genus *Methanosaeta* remained low. An increase in the levels of hydrogen-utilizing methanogens of the order *Methanomicrobiales* suggests that the major route of methane production from acetate was through a syntrophic relationship between an acetate-oxidizing organism and a hydrogen-utilizing methanogen. Thus, it was shown that anaerobic digestion of swine waste remained sustainable at total ammonia-N levels exceeding 3500 mg l^{-1} due to the ability of the hydrogen-utilizing methanogens of the order *Methanomicrobiales* to be active at these high total ammonia levels.

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